Physiological Basis for the Salicylic Acid-Mediated Tolerance of Mungbean (*Vigna radiata*) and Mustard (*Brassica juncea*)

ABSTRACT

THESIS

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SHABINA SYEED

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Physiological Basis for the Salicylic Acid-Mediated Tolerance of Mungbean (*Vigna radiata*) and Mustard (*Brassica juncea*)

Abstract

Shabina Syeed

Abstract of the thesis submitted to the Aligarh Muslim University, Aligarh, India for the degree of Doctor of Philosophy in Botany.

The present thesis comprises of six chapters.

In Chapter 1, the importance of the problem and justifications for the present work undertaken were emphasized.

Chapter 2 is the review of literature. It deals with the relevant literature on the aspects of salinity stress and salicylic acid. The chapter has been divided in sections and sub-sections for better understanding of the work of other researchers in this field of study.

Chapter 3 describes the details of the materials used in the study and methodology adopted to determine various characteristics recorded in the four experiments. Relevant information on the experimental design, location of the study and the environmental conditions during the data sampling times has been mentioned.

Chapter 4 includes the results on crop responses to treatments in the four experiments. The results were statistical analysed and the significance at $P<0.05$ was determined. The treatment means were separated by the Duncan's Multiple Range Test (DMRT).

In Chapter 5, results have been discussed in the light of observations recorded and supported with the earlier findings, if available on the subject.

undertaken, results obtained, conclusion and proposed future research are given.

Importance of the study undertaken

Salinity is a major limiting environmental factor for plant production. Around the world, 100 million ha or 5% of the arable land is adversely affected by high salt concentrations (Ghassemi *et al.*, 1995). The major causes of the soil salinity are inappropriate irrigation and the use of saline irrigation water. In dry areas, salt concentration increases in the upper soil layer due to high evaporatory water loss that exceeds precipitation (Ebert *et al.*, 2002). Attempts have been made to develop salinity stress tolerant plants. The use of salicylic acid (SA) to induce resistance of plants to abiotic stress has received considerable attention.

SA is a common plant-produced phenolic compound that can function as a plant growth regulator (Arberg, 1981). Exogenous application of SA has been reported to influence several developmental and physiological processes, i.e., seed germination (Cutt and Klessing, 1992), transpiration rate (Larque-Saavedra, 1979), stomatal closure (Rai *et al.*, 1986), membrane permeability (Barkosky and Einhellig, 1993), growth and photosynthesis (El-Tayeb, 2005). SA has also received much attention due to its role in plant responses to abiotic stresses such as ozone (Koch *et al.*, 2000), UV-B (Surplus *et al.*, 1998), heat stress (Clark *et al.*, 2004), drought (Nemeth *et al.*, 2003), oxidative stress (Shim *et al.*, 2003), salt and osmotic stress (Borsani *et al.*, 2001; El-Tayeb, 2005).

In view of the importance of SA in abiotic stress management, it was assumed that tolerant and non-tolerant cultivars of mungbean (*Vigna radiata*) and mustard (*Brassica juncea*) would respond differently to SA application and

the capacity of SA to modify tolerance in these types would be different. Therefore, the reported research was undertaken to determine whether or not applying a low concentration of SA to mungbean and mustard plants will reduce any damaging effects caused by salt stress. The response of tolerant and non-tolerant cultivars of mungbean and mustard to SA application under salt stress was determined for their growth behaviour, photosynthetic capacity, biochemical characteristics, activities of antioxidative enzymes and yield attributes.

Mungbean and mustard are important crops and grown in summer and winter season, respectively in India. Mungbean is one of the most important pulse crop and offer an excellent source of high quality of protein. Vitamin C is synthesized in sprouted seeds of mungbean with increment in riboflavin and thiamine. It is a native of India and central Asia. It is grown in these areas since pre-historic period. It is also grown in the parts of Africa and USA and has recently been introduced in Australia. In India, mungbean is grown in an area of 3 million ha with the production of about 1 million tones (www.ikisan.com). Similarly, the other test crop, the oleiferous *Brassica* is the third most important source of vegetable oil in the world after palm and soybean oil (Zhang *et al.*, 2003). The major mustard oil-producing countries include Canada, China, France, Germany, India and UK. According to a report of USDA, the world oilseed production is 397Mt in 2006-07. This total production is an increase of 9Mt, or 2% on the last season. About 90% of the total land under oilseed cultivation in India is occupied by *Brassica juncea* (Khan *et al.*, 2007).

Experimental Results

The results of the experiments are summarized below:

Experiments 1 and 2

Experiment 1 was conducted on mungbean and Experiment 2 on mustard to assess the effects of salinity stress (0, 50 and 100mM NaCl) on four

cultivars. The purpose of the study was to select tolerant and non-tolerant cultivars on the basis of their growth, photosynthetic and yield characteristics at 20 and 40DAS in mungbean and at 30, 60 and 90DAS in mustard. Yield characteristics were determined at 60DAS in mungbean and 120DAS in mustard. The design of the experiments was randomized block design. Growth characteristics determined were : root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass. Photosynthetic characteristics were : carbonic anhydrase activity and net photosynthetic rate. At harvest, yield characteristics determined were : pod length, pod number per plant, seed number per pod and seed yield.

Experiment 1 (2003): Maximum reductions in the growth and photosynthetic characteristics were noted with 100mM NaCl at 20 and 40DAS in all the cultivars of mungbean. Upto maturity stage, treatment of 100mM NaCl proved deleterious and plants did not survive in this treatment. Plants treated with 50mM NaCl, thus exhibited a significant and maximum decrease over control on yield characteristics. Therefore, 50mM NaCl concentration was considered suitable to assess tolerance of the cultivars, and this concentration was used in further experiments. The cultivar Tram exhibited maximum decrease followed by T44 whereas Pusa Vishal registered lowest decrease followed by PBM54. The order of the tolerance of the cultivars to salinity stress was Pusa Vishal > PBM54 > T44 > Tram.

Experiment 2 (2003-2004): The effect of 100mM NaCl decreased the growth and photosynthetic characteristics maximally and was more conspicuous on all the cultivars of mustard at 30, 60 and 90DAS sampling times. However, the effect of 100mM NaCl on yield characteristics was detrimental and the plants could not survive. The cultivars, Alankar and Pusa Bold had significantly more growth, photosynthetic and yield characteristics than Sakha and PBM16 under 50mM NaCl concentration. The order of the

suitability of the cultivars to salinity stress in terms of growth, photosynthetic and yield characteristics was Alankar > Pusa Bold > Sakha > PBM16.

Experiments 3 and 4

Experiments 3 and 4 were conducted based on the findings of Experiments 1 and 2. The aim of the experiments was to study the effects of exogenous application of salicylic acid in alleviating salinity stress and the physiological processes associated changes with the salicylic acid treatment on tolerant and non-tolerant cultivars of mungbean (Experiment 3) and mustard (Experiment 4). From the results of Experiment 1, Pusa Vishal and Tram cultivars of mungbean were categorized as tolerant and non-tolerant cultivars, respectively. Similarly, from Experiment 2 it was clear that Alankar and PBM16 cultivars of mustard were tolerant and non-tolerant, respectively. This has also been detailed out in earlier pages that the treatment of 100mM NaCl was deleterious for both the crops. Therefore, the plants were burned at maturity stages of the crops. Both the experiments (3 and 4) were confined with the use of 0 or 50mM NaCl for growing plants and the application of 0.0, 0.1, 0.5 and 1.0mM SA on foliage at 15DAS on tolerant and non-tolerant cultivars was studied on ameliorating salt stress effects. In these two experiments growth, photosynthetic, biochemical and yield characteristics were studied. The time of sampling for these characteristics for mungbean was 20, 40 and 60DAS, and 30, 60, 90 and 120DAS for mustard. The activities of antioxidative enzymes were also studied in both the crops at first sampling time. Growth characteristics were similar as in earlier experiments. Photosynthetic characteristics were : carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and the contents of chlorophyll and carotenoid. Biochemical characteristics were : concentration of sodium, chloride, nitrogen, phosphorus, potassium and calcium. The activities of antioxidative enzymes assayed were : catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase. Yield

characteristics were similar as in Experiments 1 and 2. The design of the experiments was randomized block design.

Experiment 3 (2004): Salt stress led to a significant reduction in growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of both the cultivars. The cultivar Tram exhibited a higher reduction than Pusa Vishal. The treatment of 50mM NaCl increased sodium and chloride concentrations in Pusa Vishal and Tram at 20 and 40DAS, and the accumulation was higher in Tram than Pusa Vishal. Exposure of plants to 50mM NaCl increased the activities of antioxidative enzymes in both the cultivars but to a higher degree in Pusa Vishal than Tram. The treatment of 0.5mM SA was found most effective in alleviating salinity stress on growth, photosynthetic, biochemical and yield characteristics. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean. In both the cultivars i.e. Pusa Vishal (tolerant) and Tram (non-tolerant) the increases were greater under non-saline (control) conditions than under saline conditions (50mM NaCl) at 20 and 40DAS. The positive effect of 0.5mM SA application was also found as it decreased sodium and chloride concentrations under both saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars further increased significantly with 0.5mM SA under both saline and non-saline conditions. In Pusa Vishal, at initial growth stage i.e. 20DAS, the application of 0.5mM SA increased potassium and calcium concentrations of plants grown under 50mM NaCl which was higher than control. However, in Tram, the increase was noted only for potassium concentration. At later growth stage, photosynthetic characteristics, nitrogen, potassium and calcium concentrations were found higher than control with the application of 0.5mM SA on Pusa Vishal plants treated with 50mM NaCl. In Tram, only potassium

concentration was found increased with 0.5mM SA of 50mM NaCl treated plants.

Experiment 4 (2004-2005): Application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations, activities of antioxidative enzymes and yield characteristics of Alankar (tolerant) and PBM16 (non-tolerant) cultivars grown under non-saline (control) conditions. Non-salinized plants treated with 0.5mM SA maintained a higher growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics than salinized plants at all the stages, indicating adverse effect of the NaCl salinity in tolerant (Alankar) as well as non-tolerant (PBM16) cultivars. Application of 0.5mM SA decreased the concentrations of sodium and chloride in both tolerant (Alankar) and non-tolerant (PBM16) cultivars, under normal and saline conditions at all the sampling times. Growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics decreased significantly with 50mM NaCl in both the cultivars but more adverse effects of salinity were found on PBM16. However, the concentrations of sodium and chloride and the activities of antioxidative enzymes increased with 50mM NaCl in both the cultivars. Application of 0.5mM SA helped to reduce the adverse effects of salinity. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl. In both the cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control at 30DAS. Nitrogen and potassium concentrations and activities of antioxidative enzymes were increased in comparison to the respective control. The application of 0.5mM SA under saline conditions also increased the calcium concentration in Alankar. At 60DAS, the treatment of 0.5mM SA on Alankar enhanced the photosynthetic characteristics, nitrogen, potassium and calcium concentrations of plants grown under 50mM NaCl. In PBM16, only two characteristics

nitrogen and potassium concentrations increased at 60DAS. At 90DAS, in Alankar, the growth and photosynthetic characteristics, nitrogen, potassium and calcium concentrations increased over control with the application of 0.5mM SA on plants treated with 50mM NaCl. In PBM16, the increase was found only for nitrogen and potassium concentrations. In Alankar, the yield characteristics were found higher than control with 0.5mM SA application on plants treated with 50mM NaCl.

The present chapter is followed by an up-to-date bibliography of the literature cited in the text.

Conclusion

It may be concluded that NaCl treatment decreased growth, photosynthetic and yield characteristics of tolerant and non-tolerant cultivars of mungbean and mustard. The salt treatment caused an accumulation of sodium and chloride to a higher extent and the essential nutrients, nitrogen, phosphorus, potassium to a lesser extent. The tolerant cultivars exhibited lesser decrease than the non-tolerant cultivars of both the crops when treated with NaCl. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean and mustard. In tolerant and non-tolerant cultivars, the increases were greater under non-saline (control) conditions than under saline conditions. The positive effect of 0.5mM SA application was found as it decreased sodium and chloride concentrations in both tolerant and non-tolerant cultivars under saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars increased significantly with SA application under both saline and non-saline conditions. Application of 0.5mM SA helped to reduce the adverse effects of salinity in both the crops. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl.

In tolerant and non-tolerant cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control for few characteristics. Salt-induced reduction in growth and finally yield characteristics in mungbean and mustard was improved by the foliar application of 0.5mM SA in both tolerant and non-tolerant cultivars. This improvement in the above characteristics due to SA was associated with improved photosynthetic capacity. The changes in net photosynthetic rate due to SA application were due to metabolic factors, other than photosynthetic pigments and leaf carotenoids. The tolerant cultivars exhibited higher growth and photosynthetic traits than non-tolerant cultivars under saline conditions, which could explain the ability of salt tolerant cultivars to show better yield characteristics under salt stress than non-tolerant cultivars. SA also maintained higher activities of antioxidative enzymes under salt stress and better synergy among the enzymes helped to reduce the active oxygen species level and damage caused by it. It may be suggested that 0.5mM SA could be used as a potential growth regulator to improve plant growth, photosynthetic and yield characteristics under salt stress.

Future Research

Salinity is a limiting environmental factor for plant production, and is becoming more prevalent in agricultural soil due to several reasons. The study reported in the thesis shows that maximum reduction in the growth and photosynthetic characteristics were noted with 100mM NaCl in all the cultivars of mungbean and mustard. The treatment of 100mM NaCl was so intense that it proved detrimental on yield characteristics and the plants could not survive. SA plays an important role in abiotic stress tolerance, and considerable interests have focused on SA due to its ability to induce a protective effect on plants under stress. Plants respond to stress by the synthesis of signaling molecules. These activate a range of signal transduction pathways. Several such signaling molecules have been identified in plants. The study of interaction of these

molecules with SA may provide fruitful information on the influence of SA on plants under normal conditions and its potential in alleviating salt stress effects. The signaling molecules may be ABA, calcium, jasmonic acid and ethylene. High salt concentration triggers an increase in levels of plant hormones such as ABA. ABA is responsible for the alteration of salt-stress genes. ABA has been found to alleviate the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates. ABA promotes stomatal closure by rapidly altering ion fluxes in guard cells under stress conditions. Other ABA actions involve modifications of gene expression, and the analysis of ABA-responsive promoters has revealed diversity of potential cis-acting regulatory elements. The nature of the ABA receptors remains unknown. The combined biophysical, genetic and molecular approaches have led to considerable progress in the characterization of more downstream signaling elements. In particular, substantial evidence points to the importance of reversible protein phosphorylation and modification of cytosolic calcium levels and pH as intermediates in ABA signal transduction. Increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term. Jasmonates also have important roles in salt tolerance. Jasmonates are generally considered to mediate signaling, such as defense responses, flowering, and senescence. However, factors involved in the jasmonate signal-transduction pathway remain unclear. Ethylene is now considered as a plant hormone regulating growth and photosynthetic responses in plants. Evidences indicate that it plays a prominent role in managing abiotic stress (Druege, 2006). Further study on the interaction of SA, ABA and ethylene may provide in-depth insight into the role of SA in alleviating salt stress.

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Dedicated

to

My parents

Dr. Nafees A. Khan
Professor of Plant Physiology

Department of Botany
Aligarh Muslim University
Aligarh - 202 002, India
Off.: 0571-702016
e-mail: naf9@lycos.com

Dated : 31.8.2007

CERTIFICATE

This is to certify that the thesis entitled, "Physiological Basis for the Salicylic Acid-mediated Tolerance of Mungbean (*Vigna radiata*) and Mustard (*Brassica juncea*)" submitted for the degree of **Doctor of Philosophy** in **Botany** is a faithful record of bonafide research work carried out at the **Aligarh Muslim University, Aligarh** by **Ms Shabina Syeed** under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

(Nafees A. Khan)

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Shabina

(Shabina Syeed)

ABBREVIATION

%	per cent
ABA	Abscisic acid
ATP	Adenosine triphosphate
BC	Before Christ
Ca	Calcium
Car	Carotenoid
CAT	Catalase
Chl	Chlorophyll
Cl	Chlorine
CO_2	Carbon dioxide
Cu	Copper
cvs.	Cultivars
d	Day
DAS	Days after sowing
DMSO	Dimethyl sulphoxide
EDTA	Ethylenediamine tetraacetic acid
ESTs	Expressed sequence tags
Fe	Iron
Fv/Fm	Photosynthetic quantum yield
g	Gravity
GA	Gibberellin
GR	Glutathione reductase
h	hour
H^+-ATPase	Hydrogen ion adenosine triphosphatase
H_2O_2	Hydrogen peroxide
HEPES	Hydroxyethylenepiperazine ethanesulfonic acid
Hsp	Heat shock proteins
K	Potassium
M	Molar
Mg	Magnesium
Min	Minute
mM	Millimolar
Mn	Manganese
mRNA	messenger Ribonucleic acid
N	Nitrogen
Na	Sodium
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate (Reduced form)
NBT	Nitroblue tetrazolium
O_2	Oxygen
$O_2^{\bullet -}$	Superoxide anion

°C	Degree Celcius
P	Phosphorus
PGRs	Plant growth regulators
pH	Hydrogen ion potential
PSII	Photosystem II
rpm	Revolutions per minute
SA	Salicylic acid
SOD	Superoxide dismutase
TAM	Tri acid mixture
t-CA	Trans-cinnamic acid
var.	Variety

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12. Effect of increasing concentrations of SA and salinity stress on net photosynthetic rate of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS). Data shown are mean ± S.E.

13. Effect of increasing concentrations of SA and salinity stress on seed yield of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) at harvest, i.e., 120 days after sowing (DAS). Data shown are mean ± S.E.

CONTENTS

INTRODUCTION

INTRODUCTION

Soil salinity is a major abiotic stress that adversely affects crop productivity. High salinity conditions have been found on the earth from the early civilizations and have been causing substantial yield reductions in agriculture throughout the world. The problem of soil salinity is increasing due to irrigation, improper drainage, seawater in coastal areas, and salt accumulation in arid and semi-arid regions. Salinity is detrimental to plant as it causes nutritional constraints by decreasing uptake of nitrogen, phosphorus, potassium and calcium, ion cytotoxicity and osmotic stress. Under salinity, ions like Na^+ and Cl^- penetrate the hydration shells of proteins and interfere with the function of these proteins. Ionic toxicity, osmotic stress and nutritional defects under salinity lead to metabolic imbalances and oxidative stress (Hirt and Shinozaki, 2004).

It has been reported that about one-third of world-irrigated land is affected by soil salinity (El Saidi, 1997). The United Nations Environment Program estimates that 20% of the agricultural land and 50% of the crop land in world is salt-stressed (Flowers and Yeo, 1995).

Around the world, 100 million ha or 5% of the arable land is adversely affected by high salt concentrations (Ghassemi *et al.*, 1995). In India about 7-12 million hectares of land are known to have been degraded by salinity with varying degrees of salt accumulations. The problem is acute in the semi-arid and arid tracts of Indo-Gangetic alluvial plains where about 40% of the total affected area is concentrated (Agarwal *et al.*, 1979). Besides, an additional area of about 15-20 million hectares of land in canal irrigated tracts runs the risk of being degraded through the influence of salts (Abrol, 1986). The major causes of the soil salinity are inappropriate irrigation and the use of saline irrigation water. In dry areas, salt concentration increases in the upper soil layer due to high evaporatory water loss that exceeds precipitation (Ebert *et al.*, 2002).

Based on the capacity of plants to grow under high salt conditions, they are classified as halophytes or glycophytes. The halophytes grow in saline soils in high concentrations of salts, while glycophytes can not grow in the presence of high salt concentrations. Halophytes can withstand salts that are more than twice the concentration of sea water.

Plant salt tolerance is necessary for sustainable food production on marginal lands and to potentially improve overall crop yield. Plant salt tolerance mechanisms can be grouped into cellular homeostasis (including ion homeostasis and osmotic adjustment), stress damage control (repair and detoxification and growth regulation) (Hirt and Shinozaki, 2004). As halophytes can tolerate high salt concentrations the inherent mechanism and processes may be used as a tool for incorporating tolerance in other plants. In addition to halophytes other plant types also have biochemical and physiological strategies for salt tolerance. These mechanisms can be studied and enhanced in the plant types that are not tolerant to salt stress. These mechanisms are : (i) control of ion uptake and accumulation in leaves (ii) synthesis of compatible osmolytes (iii) alteration in photosynthetic pathway (iv) induction of antioxidative enzymes and (v) induction of phytohormones (Parida and Das, 2005).

Under salt stress, plants adapt several mechanisms to protect themselves. A wide range of second messengers have been implicated to a variety of stresses. Several techniques for either alleviating the salt effects or inducing salt tolerance by manipulating soil amendments, plant breeding, methods of sowing, and management practices have been tried but not much success has been achieved so far and the problem continues to be a serious limiting crop yields. Salinity is known to disturb the endogenous hormonal balance in plants. In general, the levels of growth promoters are reported to decrease and of inhibitors to increase under saline conditions (Levitt, 1980). As these plant regulators are known to control internal metabolism, attempts need

be made to explore the possibility of using these for alleviating salt stress-induced physiological effects. Plant growth regulators are effective in reducing the adverse effects of salinity. Presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Dara, 1971). Concerned attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators (Datta *et al.*, 1998). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be reduced to some extent by treating seeds with the proper concentration of a suitable hormone (Darra *et al.*, 1973). Studies have demonstrated that salicylic acid (SA) induces tolerance to salinity stress with altered water relations and consequent changes in solute concentration (Senaratna *et al.*, 2000, 2003). Senaratna *et al.* (2007) have reported that SA application induces salinity tolerance in a variety of genetically diverse plant taxa.

SA is a common plant-produced phenolic compound that can function as a plant growth regulator (Arberg, 1981). Exogenous application of SA has been reported to influence several developmental and physiological processes, i.e., seed germination (Cutt and Klessing, 1992), transpiration rate (Larque-Saavedra, 1979), stomatal closure (Rai *et al.*, 1986), membrane permeability (Barkosky and Einhellig, 1993), growth and photosynthesis (El-Tayeb, 2005). SA has also received much attention due to its role in plant responses to abiotic stresses such as ozone (Koch *et al.*, 2000), UV-B (Surplus *et al.*, 1998), heat stress (Clark *et al.*, 2004), drought (Nemeth *et al.*, 2003), oxidative stress (Shim *et al.*, 2003), salt and osmotic stress (Borsani *et al.*, 2001; El-Tayeb, 2005).

The SA pathway, thus considered to be a key factor in inducing tolerance. SA was first discovered as a major component in the extracts from Salix (Willow) whose bark from ancient times has been in use as an anti-

inflammatory drug. This acid is phenol, ubiquitous in plants generating a significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport. It also plays a role in thermogenesis in lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity (Raskin, 1992). SA has been found in signal regulation and gene expression in the course of leaf senescence in *Arabidopsis* (Morris *et al.*, 2000), inhibitor of fruit ripening (Srivastava and Dwivedi, 2000) and of other processes.

During the last two decades this substance has drawn attention of researchers due to its ability to induce systemic acquired resistance in plants to different pathogens, which is manifested in the appearance of pathogenesis related proteins. SA is considered to serve as a signal in the induction of expression of these genes (Metraux, 2001). At present, considerable interest has been generated in SA due to its ability to produce a protective effect on plants under abiotic stress. The application of SA has been found to increase tolerance of wheat and maize seedlings to salinity (Arfan *et al.*, 2007; Gunes *et al.*, 2007), water deficit (Bezrukova *et al.*, 2001), of tomato and bean plants to low and high temperature (Senaratna *et al.*, 2000) as well as of heavy metals of rice plants (Mishra and Choudhuri, 1999; Choudhury and Panda, 2004).

In view of the importance of SA in abiotic stress management, it was assumed that tolerant and non-tolerant cultivars of mungbean (*Vigna radiata*) and mustard (*Brassica juncea*) would respond differently to SA application and the capacity of SA to modify tolerance in these types would be different. Therefore, the reported research was undertaken to determine whether or not applying a low concentration of SA to mungbean and mustard plants will reduce any damaging effects caused by salt stress. The response of tolerant and non-tolerant cultivars of mungbean and mustard to SA application under salt stress was determined for their growth behaviour, photosynthetic capacity,

biochemical characteristics, activities of antioxidative enzymes and yield attributes.

Mungbean and mustard are important crops and grown in summer and winter season, respectively in India. Mungbean is one of the most important pulse crop and offer an excellent source of high quality of protein. Vitamin C is synthesized in sprouted seeds of mungbean with increment in riboflavin and thiamine. It is a native of India and central Asia. It is grown in these areas since pre-historic period. It is also grown in the parts of Africa and USA and has recently been introduced in Australia. In India, mungbean is grown in an area of 3 million ha with the production of about 1 million tones (www.ikisan.com). Similarly, the other test crop, the oleiferous *Brassica* is the third most important source of vegetable oil in the world after palm and soybean oil (Zhang *et al.*, 2003). The major mustard oil-producing countries include Canada, China, France, Germany, India and UK. According to a report of USDA, the world oilseed production is 397Mt in 2006-07. This total production is an increase of 9Mt, or 2% on the last season. About 90% of the total land under oilseed cultivation in India is occupied by *Brassica juncea* (Khan *et al.*, 2007).

Keeping in view the importance of SA on physiological activities of plants and in amelioration of stress, the reported work was undertaken with the following objectives.

1. To screen and select salt tolerant and non-tolerant cultivars of mungbean and mustard.
2. To study the effects of exogenous application of salicylic acid in alleviating salinity stress and physiological and biochemical changes associated with the salicylic acid application triggering salt tolerance.

REVIEW OF LITERATURE

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2.1 Introduction

The cultivation of plants was one of the greatest revolutionary accomplishments that presumably began in the Mesolithic or Middle stone age from 12000 to 6000 BC, when man lived with spear, bow and fishing net. Since then the increasing needs urged him to search for better techniques to fulfil his requirements. At the present age of 'Space age', agriculture faces many challenges worldwide due to interaction of several phenomenon, important being 'population explosion' and changing environmental conditions.

Agriculture in the advent of 'Green Revolution' was dominated by the efforts of increasing productivity, but it now needs multiple objectives. The soil condition and crop production occupy priority among others. Our dependence on chemical fertilizers, use of waste water for irrigation led plants to face a variety of abiotic and biotic environmental stresses. Salinity of soil is a major abiotic stress. It is the first chemical stress factor encountered during the evolution of life on earth because earliest living organisms were marine forms (Jacobsen and Adams, 1958).

Soil salinity predates human civilization and is probably a cause of the breakdown of the ancient Sumerian civilization (Jacobsen and Adams, 1958). Saline soil is characterized by toxic levels of chlorides and sulfates of sodium. The electrical conductivity (EC) of saturation extracts of saline soil is > 4mmhos cm^{-1}, exchangeable Na percentage is less than 15 and pH is less than 8.5 (Marschner, 1995). Salinity is detrimental to plants as it causes various kinds of alterations such as (i) nutritional constraints by decreasing uptake of phosphorus (P), potassium (K), nitrogen (N) and calcium (Ca) (ii) ion toxicity mainly due to Na^+, Cl^- and SO_4^{--} and (iii) osmotic stress: Na^+ competes with K^+ in biochemical reactions, which is inimical to cellular processes. Under

salinity, ions like Na^+ and Cl^- penetrate the hydration shells of proteins and interfere with the non-covalent interactions between amino acids of proteins. This leads to conformational changes and loss of function of proteins. In addition, ion toxicity, osmotic stress and nutritional defects under salinity may lead to metabolic imbalances causing oxidative stress (Zhu, 2001).

In arid and semi-arid areas of India, crop production is limited because of soil-salinity and/or-alkalinity. It has been estimated that about 7-12 million hectares of land in the country have either gone out of cultivation or this area produces low yields of crops (Agarwal, 1979).

2.1.1 Non-saline alkali or sodic soils

These soils do not contain any large amount of neutral salts and, as such, the electrical conductivity is < 4mmhos cm^{-1}. The detrimental effect of alkali soil on plants is largely due to toxicity of high amount of exchangeable sodium and the pH. Alkali soils have an exchangeable sodium percentage of more than 15 and pH greater than 8.5. Such soils have low infiltration rate and the physical condition is unfavorable. Because of high alkalinity, resulting from sodium carbonate, the surface soil is discoloured and black, and hence the term black alkali is frequently used to designate the non-saline alkali soil.

2.1.2 Saline alkali soils

This group of soils is both saline and alkali. They have appreciable amounts of soluble salts, as indicated by the values of electrical conductivity which are > 4mmhos cm^{-1}. Also, the exchangeable sodium percentage is greater than 15. The pH, however, is likely to be less than 8.5.

2.1.3 Causes of salinity

During the periods of higher than average rainfall, the soluble salts are leached from the more permeable high-lying area to the low-lying areas. This results in the accumulation of salts if the drainage is poor. Moreover, the excessive irrigation of the uplands with water containing salts also results in the accumulation of salts. In areas having a salt layer at lower depth in the profile,

seasonal irrigation may favour the upward movement of the salts. Rise in the water table within 2m of the surface due to irrigation, the obstruction of natural drainage may also cause soil salinity because of developmental activities, e.g. roads and canals and the situation of natural drainage. In the coastal areas, the ingress of seawater induces salinity in the soil.

2.2. Why salinity a major problem?

Substantial areas of the earth's potentially productive lands are affected by soil salinity and alkalinity. Ponnamperuma (1977) estimated that there are 381 million hectares of saline soils and the problem is increasing because of inadequate irrigation and drainage practices. The agronomic problem of salinity is compounded by the relatively low salt tolerance of many of the major crop plants (Maas and Hoffman, 1977). High concentration of soluble salts in the top soil layer is detrimental to profitable agriculture. In India about 7 million hectares of land are known to have been degraded by salinity/alkalinity with varying degrees of salt accumulations. According to some estimates, the total salinity/alkalinity-affected area is around 12 million hectares. The problem is acute in the semi-arid and arid tracts of Indo-Gangetic alluvial plains where about 40% of the total affected area is concentrated (Agarwal *et al.*, 1979). Besides, an additional area of about 15-20 million hectares of land in canal irrigated tracts runs the risk of being degraded through the influence of salts (Abrol, 1986). The increasing salt-affected land in India has been estimated to be around several hundred Km^2 a year (Flowers *et al.*, 1977).

2.3 Effects of salinity stress on plant development

2.3.1 Salinity and growth characteristics

The effect of salinity on plant growth is a complex syndrome that involves osmotic stress, ion toxicity, and mineral deficiencies (Neumann, 1997; Yeo, 1998; Hasegawa *et al.*, 2000; Munns, 1993, 2002). Several factors may contribute to the reduction in growth exhibited by plants under salinity stress. One significant factor may be related to inhibition of vascular tissue production

under stress (Shininger, 1979; Ewing, 1981). Sepehr *et al.* (2003) reported that with increasing salinity levels, leaf area, shoot and root dry matter and shoot and root fresh matter of *Zea mays* plants decreased. Muhling and Lauchli (2003) found no differences in growth between the genotypes *Lophopyrum elongatum* (Host) A. Love (Salt tolerant) and *Triticum aestivum* L. cv Chinese Spring (Salt sensitive) under moderate salinity of 75mM NaCl. However, other studies involving severe stress of 100 and 200mM NaCl, *Lophopyrum elongatum* was found more tolerant than the other genotypes (Colmer *et al.*, 1995; Santa-Maria and Epstein, 2001). Cherian and Reddy (2000) reported considerable enhancement in fresh and dry mass accumulation in halophyte *Sueada nudiflora* Moq. with the increase in salinity from 0-680mol m^{-3} NaCl. However, dry mass accumulation was not uniform in all plant parts. Root dry mass decreased with the increase in salinity, whereas plant water content and fresh mass increased with salinity. Growth stimulation at low to moderate external salinity has been reported for many halophytes (Flowers *et al.*, 1977; Reddy *et al.*, 1992; Al-Zaharani and Hajar, 1998). The observed growth response was more or less consistent with the natural distribution of this species in marshy and island waters where salinity exceeds more than 500mol m^{-3}.

Contrarily, Sudhakar *et al.* (1990), Brugnoli and Malco (1991), Sanchez *et al.* (1997) and Sultana and Itoch (2000) observed decrease in growth and dry mass accumulation due to salinity stress. Gossett *et al.* (1994) reported that 150mM NaCl was the minimum salt concentration that produced significant differences in growth reduction between the more salt tolerant and less salt tolerant cultivars. Although 150mM NaCl significantly reduced total leaf area and fresh mass of all cultivars and there was a significant salinity × cultivar interaction, growth reduction in the salt tolerant cultivars was less severe than the salt sensitive cultivars. Gossett *et al.* (1992) also reported that the growth reduction in the salt sensitive cultivars was significantly greater than the salt

tolerant cultivars. Bandeoglu *et al.* (2004) reported that growth of lentil plants, estimated as shoot-root length, leaf area and fresh-dry mass, was significantly reduced by NaCl treatments. At 200mM salinity stress, more growth retardation was observed in leaf tissues when compared to root tissues. Hernandez *et al.* (1999) reported a reduction in the growth of pea plants subjected to 0-160mM NaCl stress. Similarly, in rice leaves under higher saline conditions, relative growth rate was decreased in salt sensitive cultivar whereas salt tolerant cultivars exhibited no significant change (Dionisio-Sese and Tobita, 1998). Salinity can affect growth and dry mass accumulation of rice (Sultana *et al.*, 1999; Asch *et al.*, 2000). Sultana *et al.* (2001) reported that seawater salinity significantly decreased tiller number, leaf area and top dry mass of rice. In some recent reports, the effects of salinity have been described on the basis of plant tolerance to salinity. Li *et al.* (2005) observed that black seeds of *Suaeda salsa* were more sensitive to salt in comparison to brown seeds. Brown seeds absorbed water more quickly in comparison to the black seeds and were found to be more tolerant to salt stress. In another study, Bouhmouch *et al.* (2005) compared the salt tolerance of five *Phaseolus vulgaris* cultivars differing in seed colour, grown on nitrates and different concentrations of NaCl. The cultivar Coco Blanc was found most sensitive and SMV29-21 as most tolerant.

Shaikh *et al.* (2007) found inhibition of seed germination in *Urochondra setulosa* with the increase in salt concentration, with few seeds germinated at and above 400mmol l^{-1} concentration. Ksouri *et al.* (2007) observed reduction in shoot biomass and leaf expansion at 100 and 400mM NaCl in *Cakile maritima Tunisian* accessions Tabarka and Jerba. Tuna *et al.* (2007) found that tomato (*Lycopersicon esculentum* Mill.) cv. Target F1 grown under salt stress produced low dry matter, fruit mass, and relative water content. Fornes *et al.* (2007) found that *Petunia hybrida* and *Calceolaria hybrida* ornamentals were tolerant to salinity. Saline-treated *Petunia* plants reduced their growth slightly

and increased N and chlorophyll contents in the leaves. *Calceolaria* experienced a strong reduction in growth and a delay in flowering but no toxicity symptoms and mortality were recorded. These two species were moderate NaCl accumulators. The other ornamental *Calendula officinalis* was sensitive to salinity; 16% of the plants died and the survived ones experienced a heavy reduction of growth, a decrease in chlorophyll and a large accumulation of salts in the leaves.

2.3.2 Salinity and photosynthetic characteristics

Several studies have shown that salinity affected photosynthesis by affecting photosynthetic characteristics. In the following pages a brief account of some studies showing effects of salinity on photosynthetic characteristics have been described. Khan and Panda (2003) reported a decrease in chlorophyll *a*, chlorophyll *b* and total chlorophyll content and an increase in carotenoid content with the increase in the NaCl concentrations in aquatic weed (*Spirodela polyrhiza*). Similar findings were also reported for some mesophytic plants (Misra *et al.*, 1997; Singh and Singh, 1999). Panda and Khan (2003) reported that in 13 and 14d old rice leaves, chlorophyll and carotenoid content decreased uniformly with an increase in NaCl and $CaCl_2$ salt concentrations which were higher in NaCl treatment, suggesting an inhibition of photosynthetic efficiency in salt sensitive rice plant in the presence of NaCl and $CaCl_2$ stress. In bean (*Phaseolus vulgaris* L.), a salt sensitive species, and cotton (*Gossypium hirsutum* L.), a salt tolerant species, the reduction in assimilation was found to be mostly due to stomatal limitation (Brugnoli and Lauteri, 1991), whereas other authors ascribed the reduction in photosynthesis to non-stomatal limitation (Dunn and Neales, 1993). Meloni *et al.* (2003) reported that the net photosynthetic rate of cotton cultivars, Guazuncho and Pora was significantly inhibited by NaCl salinity. The increase in NaCl stress significantly decreased the stomatal conductance of both cotton cultivars. In Guazuncho, all NaCl treatments led to reduction in the contents of total chlorophyll. In Pora,

however, there were no reductions in total chlorophyll caused by NaCl stress. The initial fluorescence and the quantum yield of PSII, as indicated by Fv/Fm in the dark, were not affected by salt treatments in both cotton cultivars. In glycophytes, the inhibition of photosynthesis under salinity stress has been attributed to stomatal closure (Steduto *et al.*, 2000), although direct salt effects on several biochemical and photochemical processes have been also reported (Chen *et al.*, 1999; Sultana *et al.*, 1999). Decreases in stomatal conductance and net photosynthetic rate due to NaCl salinity have been reported for cotton (Brugnoli and Lauteri, 1991). Delfine *et al.* (1999) reported no changes in the chlorophyll content in 20d salt stressed spinach (*Spinacia oleracea* L.) plants. The leaf photochemistry was resistant to salt stress as determined by Brugnoli and Bjorkman (1992) in cotton and by Delfine *et al.*, (1999) in spinach. Delfine *et al.* (1999), however, found that the photochemical efficiency of the salt-stressed leaves of spinach reduced after 50d salt stress, indicating that high salt concentrations started to affect leaf photochemistry. Divate and Pandey (1980) reported that chlorophyll contents and the net photosynthetic rate decreased with the increase in salt concentrations in grapes. Similar results for chlorophyll content have also been reported by Kanwar and Bhambota (1968) and similar reduction in the net photosynthetic rate under high levels of salinity has also been demonstrated by various workers (Wadleigh and Ayers, 1945; Boyer, 1965; Gale *et al.*, 1967). Various salinity treatments have been found stimulating the rate of respiration per unit of leaf area. The greatest stimulation was observed at the highest level of salt concentration. Sultana *et al.* (2001) reported that salt stress led to a significant inhibition of leaf net photosynthetic rate of rice plants. Net photosynthetic rate was found decreased when concentration of seawater increased from 8.8 to 35%. The increase in the salinity significantly decreased stomatal conductance of leaves, whereas internal CO_2 partial pressure was not greatly affected. Leaf water potential, osmotic potential and relative leaf water content significantly decreased with

the increasing concentrations of salinity (Delfine *et al.*, 1999; Sultana *et al.*, 1999). Salt stress has been reported to decrease photosynthesis through stomatal and non-stomatal factors (Yeo *et al.*, 1985; Sharma and Hall, 1991; Dionisio-Sese and Tobita, 2000). Responses of chlorophyll a fluorescence to salinity have been studied in barley (Larcher *et al.*, 1990; Belkhodja *et al.*, 1994) and sorghum (Sharma and Hall, 1991, 1992; Lu and Zhang, 1998). Larcher *et al.* (1990), Brugnoli and Lauteri (1991), Mishra *et al.* (1991), Jimenez *et al.* (1997) and Belkhodja *et al.* (1994, 1999) reported no significant change in the photosynethetic quantum yield (Fv/Fm) in response to NaCl treatments. However, Smillie and Nott (1982), Bongi and Loreto (1989) and Misra *et al.* (2001) suggested Fv/Fm as an early indicator of salt stress. There was little impact of salt on CO_2 assimilation rates at moderate NaCl concentrations reported for various varieties of sorghum (Sharma and Hall, 1991; Masojidek and Hall, 1992; Nagy *et al.*, 1995). The sorghum response to NaCl was found similar to *Phaseolus vulgaris* L. (Seeman and Critchley, 1985; Brugnoli and Lauteri, 1991), *Triticum aestivum* L. and *Hordeum vulgare* L. (Rawson, 1986; Sharma and Hall, 1991). A positive correlation between stomatal conductance and CO_2 assimilation rate has been suggested, and stomatal conductance as the primary factor limiting photosynthesis under salt stress. Stomatal factors are generally more significant at medium salinity and non-stomatal limitations are more relevant at high salinity (Everard *et al.*, 1994). Reddy and Vora (1986) reported that salinity could affect chlorophyll concentration of leaves through inhibition of synthesis of chlorophyll or an acceleration of its degradation. Data from intact leaves of sorghum (Lu and Zhang, 1998) and other plant species (Larcher *et al.*, 1990; Mishra *et al.*, 1991; Belkhodja *et al.*, 1994) were interpreted to indicate that salinity did not affect the Fv/Fm ratio. Contradictory results have been reported for rice, mungbean and mustard seedlings (Lutts *et al.*, 1996; Dionisio- Sese and Tobita, 2000; Misra *et al.*, 2001). These results imply that effects of salt on potential

photochemical efficiency of PSII might be species specific. Lu and Zhang (1998) found PSII to be highly resistant to salinity stress. Salinity has been found to affect reaction centers of PSII either directly (Masojidek and Hall, 1992) or via an accelerated senescence (Hasson and Poljakoff-Mayber, 1981; Kura-Hotta *et al.*, 1987). Omami and Hammes (2006) found net photosynthetic rate and stomatal conductance reduced under salt stress treatments but photosynthetic water-use efficiency increased.

Cramer *et al.* (2007) found that salinity affected a higher percentage of transcripts involved in transcription and protein synthesis in grapevine. Neocleous and Vasilakakis (2007) described the effect of increasing salt concentration in red raspberry (*Rubus idaeus* L.). Salt stress declined net photosynthetic rate, stomatal conductance, leaf chlorophyll content, fluorescence and transpiration rate. The intercellular CO_2 and water-use efficiency remained unaffected.

2.3.3 Salinity and biochemical characteristics

A decrease in Na^+: K^+ ratio was recorded for aquatic weed *Spirodela polyrhiza* tissues which proved to be a salt tolerant, with an avoidance of Na^+ toxicity by better K^+ levels in the tissue (Qadar, 1991). Sepehr *et al.* (2003) reported that concentrations of Ca, Mg and K in treated plants of *Zea mays* were decreased by salinity. An observation similar in lines was also made by Lynch and Lauchli (1985) and Ouzounidou *et al.* (1997). Salinity induced Na-transporters were found increased more in roots than shoots (Hasegawa *et al.*, 2000). Ehret *et al.* (1990) found that mineral nutrition of non-halophytes as influenced by the presence of salt was a consequence of ion interactions and salinity induced low calcium levels in plants or calcium deficiency.

Numerous metabolic changes have been noted to occur in plants exposed to ionic stress, e.g. Na^+/H^+ exchange processes are activated so that K^+ can pass across the cell membrane (Watad *et al.*, 1986) and Na^+ can be pumped into tonoplasts (Binzel *et al.*, 1988; Garbarino and DuPont, 1989). An

osmotically regulated gene *salT*, encodes a protein whose mRNA accumulates in sheaths and roots of rice seedlings exposed to salt stress and correlates with patterns of Na^+ accumulation during salt stress (Claes *et al.*, 1990). Apoplastic proteins in leaves have been found accumulated under salinity (Ramanjulu *et al.*, 1999). A significant increase in apoplastic proteins was observed in leaves of moderately salt tolerant barley at the concentration of 10mM NaCl. Muhling and Lauchli (2003) reported that intracellular Na in leaves of *Lyphopyrum elongatum* (Host) A. Love (salt tolerant) and *Triticum aestivum* L. cv. Chinese Spring (salt sensitive) wheat genotypes increased under salinity. No significant differences in Na and K were found in leaves and roots among the genotypes under NaCl salinity. These authors found a lower accumulation of Na and enhanced accumulation of K in younger leaves of the salt tolerant genotype and consequently higher K/Na ratio under 100 and 200mM NaCl. No differentiation was made between older and younger leaves at a moderate salinity level of 75mM NaCl. The Ca in leaves of the salt sensitive wheat genotype decreased under NaCl salinity. This suggested that Ca homeostasis in the leaves was affected under NaCl stress. It was found that Na displaced membrane-associated Ca (Cramer *et al.*, 1985; Lynch *et al.*, 1987; Lauchli and Schubert, 1989) which might lead to higher Ca in the leaf apoplast. Higher plasma membrane permeability under NaCl salinity (Cramer *et al.*, 1985; Lynch *et al.*, 1987) leads to a stimulated K efflux into the leaf apoplast. In fact, apoplastic K in leaves of the salt sensitive wheat genotype increases under salinity which indicates that Na affects the function of the plasma membrane in leaves of the salt sensitive wheat genotype. Higher protein concentration in intercellular leaf compartments of both genotypes under salinity supported by the investigation of Ramanjulu *et al.* (1999) who found increased protein in leaf apoplast at low NaCl (10mM) salinity. Another study shows that salinity stress does not elicit changes in protein pattern in leaves of salt sensitive wheat compared to more salt tolerant barley (Ramagopal, 1987). Polypeptide

expression has also been found significantly enhanced under salinity. A 15KDa polypeptide was significantly increased in the intracellular leaf compartment under salinity. Cherian and Reddy (2000) reported that the inherent capacity to accumulate large quantities of ions from the external medium probably formed the basis for the extended growth stimulation at higher salinities in *S. nudiflora*. The increase in NaCl concentration gradually increased Na^+ accumulation and the accumulation of Na^+ was significantly higher in leaves than in shoot or root. Similarly, chloride concentration increased significantly with external salinity in all plant parts, but showed a slight decrease at 680mol m^{-3} NaCl. Salinity treatment decreased concentration of K^+ in leaf. However, in shoot and root, the levels of K^+ were higher. The accumulation of Na^+ and Cl^- was significantly higher than K^+. Na^+/K^+ ratio increased steadily with salinity. The findings that high concentrations of Na^+ found in *S. nudiflora* leaves were in agreement with the findings of Eshel (1985) in *S. aegyptiaca*. Potassium has been reported as a major osmoticum in plant cells under high salt conditions (Epstein, 1998). Externally supplied Ca^{2+} has been shown to ameliorate the adverse effect of salinity in plants, presumably by facilitating higher K^+/Na^+ selectivity (Hasegawa *et al.*, 2000). The increased calcium content in the nutrient solution also ameliorates the inhibitory effect of NaCl (Epstein, 1998).

The toxic effects of excess accumulations of Cl^- in leaf tissue have been well documented. Greenway and Munns (1980) and Seeman and Sharkey (1986) noted that excessive Cl^- caused a reduction in ribulose–1,5–bisphosphate regeneration capacity in *Phaseolus vulgaris* L. and suggested that this might be due to the effect of the Cl^- ion on the capacity for ATP formation. In cotton, Plaut and Federman (1991) reported that salt stress caused slight reduction in K^+ concentration, without any change in Ca^{2+} and Mg^{2+}concentrations, but 3-6 fold increases in Na^+ and Cl^- concentrations. Maas and Grieve (1987) demonstrated that high NaCl treatment induced Ca^{2+} deficiency symptoms in corn, presumably due to change in membrane integrity

at high Na^+/Ca^{2+} ratio. Ionic imbalance occurred in the cells due to excessive accumulation of Na^+ and Cl^- and reduced the uptake of other mineral nutrients, such as K^+, Ca^{2+} and Mn^{2+} (Cramer and Nowak, 1992; Khan *et al.*, 1997; Lutts *et al.*, 1999).

Regulation of ion transport is one of the important factors responsible for salt tolerance of plants. Na^+ moves passively through cation channel from the saline growth medium into the cytoplasm of plant cells (Marschner, 1995; Jacoby, 1999; Mansour *et al.*, 2003). Salt tolerance in plants is generally associated with low uptake and accumulation of Na^+. It has been reported that overexpression of the vacuolar Na^+/H^+ antiporter that sequesters Na^+ in vacuoles improved the salinity tolerance in *Arabidopsis*, tomato, and brassicas (Aharon *et al.*, 2003). Similarly, overexpression of plasma membrane Na^+/H^+ antiporter gene (SDSI) improved salt tolerance in *Arabidopsis thaliana* (Shi *et al.*, 2003). Janicka-Russak and Klobus (2007) investigated that in cucumber roots treatment of plants with salt distinctly increased the activity of the plasma membrane H^+-ATPase as well as of the vacuolar H^+-ATPase. Zheng *et al.* (2006) reported that of 190 maize expressed sequence tags (ESTs) 36ESTs in leaves and 41ESTs in roots were significantly up-regulated by high-salinity stress. Diedhiou and Golldack (2006) found that OsCLC1 transcript levels were repressed in leaves and roots of the salt sensitive Cl^- accumulating rice line IR29 in response to salt stress. However, in salt tolerant type, Pokkali expression was transiently induced. Under the same conditions, in IR29 mRNA levels of the Na^+/H^+ antiporter OsNHX1 and of the vacuolar H^+-ATPase subunit OsVHA-B decreased upon salt stress whereas Pokkali showed transient stimulation of OsVHA-B transcripts.

2.3.4 Salinity and oxidative stress

There is now conclusive evidence that production of active oxygen species (AOS) is enhanced in plants in response to different environmental stresses such as salinity, drought, water-logging, temperature extremes, high

light intensity, herbicide treatment or mineral nutrient deficiency (Wise and Naylor, 1987; Monk and Davies, 1989; Cakmak and Marschner, 1992; Gossett *et al.*, 1994; Mittova *et al.*, 2000,2002). Plants containing high concentrations of antioxidants have been shown to possess considerable resistance to the oxidative damage caused by the activated oxygen species (Wise and Naylor, 1987; Spychalla and Desborough, 1990; Shalata and Tal, 1998; Garratt *et al.*, 2002).

The active oxygen species such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are produced during normal aerobic metabolism when electrons from the electron transport chains in mitochondria and chloroplasts are leaked and react with O_2 in the absence of other acceptors (Halliwell and Gutteridge, 1985; Thompson *et al.*, 1987). However, plants generally have the ability to eliminate superoxide with the help of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen, and is important in preventing the reduction of metal ions and hence the synthesis of hydroxyl radicals. Hydrogen peroxide can be eliminated by an ascorbate peroxidase (APX) located in the thylakoid membrane (Chen and Asada, 1989).

Comparing the mechanisms of antioxidant production in salt tolerant and salt sensitive plants, Dionisio-Sese and Tobita (1998) reported a decline in SOD activity and an increase in peroxidase activity in the salt sensitive rice varieties, Hitomebore and IR28 in response to salt stress. These salt sensitive varieties also showed an increase in lipid peroxidation and electrolyte leakage as well as Na^+ accumulation in the leaves under saline conditions. In contrast, two salt tolerant rice varieties, Pokkali and Bankat, showed differing protective mechanisms against AOS under salt stress. Rice *cv.* Pokkali showed only a slight increase in SOD but a slight decrease in peroxidase activity, and almost unchanged lipid peroxidation, electolyte leakage and Na^+ accumulation under saline conditions. The other cultivar Bankat showed Na^+ accumulation in

leaves and symptoms of oxidative damage similar to the salt sensitive cultivars. In cotton, significantly higher constitutive concentrations of catalase (CAT) and α-tocopherol were found in salt tolerant than in salt sensitive lines (Gossett *et al.*, 1994, 1996). Salt stress caused a considerable increase in the activities of peroxidase and glutathione reductase (GR) in the salt tolerant cultivars, whereas the activities of these enzymes remained unchanged or decreased in the salt sensitive cultivars. The salt tolerant cultivars also had a lower oxidized/reduced ascorbate ratio and a higher reduced/oxidized glutathione ratio than the salt sensitive lines under saline conditions. Lipid peroxidation in the salt sensitive lines increased more in salt tolerant lines under salt stress. The authors were of the view that high levels of antioxidants and an active ascorbate-glutathione cycle are associated with salt tolerance in cotton (*Gossypium herbaceum*). Garratt *et al.* (2002) found that SOD and GR activities were higher in the cultured cells of salt tolerant cv. Dhumad than in those of moderately tolerant (H-14) or salt sensitive (Rahs-2) cultivars in saline medium. Shalata and Tal (1998) assessed the possible involvement of the antioxidant system in the salt tolerance of cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii*. They reported that in the latter species the constitutive level of lipid peroxidation and the activities of catalase and glutathione reductase were lower, whereas the activities of superoxide dismutase, ascorbate peroxidase, and dehydroascorbate reductase were inherently higher than those in the cultivated tomato species. Working with the same two species of tomato, Mittova *et al.* (2000) concluded that high salt tolerance of the wild salt tolerant species was due to maintenance of high SOD to ascorbate peroxidase activity. In another study, Mittova *et al.* (2002) found that compared with cultivated tomato (*Lycopersicon esculentum*), the better protection of wild salt tolerant tomato (*L. pennellii*) root plastids from salt induced oxidative stress was correlated with increased activities of SOD, ascorbate peroxidase and guiacol peroxidase.

Studies using modern genetic engineering techniques, Foyer *et al.* (1994), Van Camp *et al.* (1994), Foyer *et al.* (1995), and Polidoros and Scandalios (1999) showed an improvement in the salt tolerance in several crops through the over expression of specific enzymes for scavenging active oxygen species.

Molina *et al.* (2002) have reported higher glutathione reductase and ascorbate peroxidase activities in NaCl-adapted tomato cells and suggested their role in higher tolerance to NaCl-induced oxidative stress. Porcel *et al.* (2003) attributed higher glutathione reductase activity in roots and nodules of mycorrhizal soybean plants under drought stress to decreased oxidative damage to biomolecules, which were involved in premature nodule senescence. Dalmia and Sawhney (2004) reported that dehydroascorbate reductase showed a linear increase in its activity with various stress levels in wheat seedlings. A similar trend was exhibited by glutathione reductase. Higher level of stress has been found associated with lesser increase in ascorbate peroxidase and catalase (Egert and Tevini, 2002). Though the activities of dehydroascorbate reductase and glutathione reductase increased steadily with stress level, it appeared they were insufficient to maintain the redox status of glutathione and ascorbate pools due to the increased levels of active oxygen species and the seedlings became increasingly vulnerable to damage by oxidative stress.

The increase in total peroxide content and lipid peroxidation measured in terms of malondialdehyde content in the plant tissue under NaCl treatment may be ascribed as oxidative damage. The increase in peroxide content with a concomitant increase in lipid peroxidation suggests a salinity stress-mediated membrane distortion (Shalata and Tal, 1998; Khan and Panda, 2002). Panda and Khan (2003) reported that there was a uniform increase in total peroxide content with the increase in salt concentration in 13 and 14d old rice leaves in NaCl and $CaCl_2$ salt treatments with a maximum increase in 14d old leaves suggesting an accumulation of a peroxide content with the aging.

Lopez-Gomez *et al.* (2007) reported an increase in monodehydro-ascorbate reductase, polyphenol oxidase and decrease in superoxide dismutase in loquat plants (*Eriobotrya japonica* Lindl.) subjected to higher salt treatment. Rodriguez *et al.* (2007) observed that superoxide anion ($O_2^{\bullet-}$) production was inhibited by 50 and 100mM NaCl in maize. Inhibitory effects of NaCl and reduced Ca^{2+} supply were also observed in gel assessment of $O_2^{\bullet-}$ generating activity.

2.3.5 Salinity and yield characteristics

Although plant breeders have successfully improved salinity tolerance of some crops in recent years using seed yield as the main selection criteria. However, the selection could be more convenient and practicable if the crop possesses distinctive indicators of salt tolerance at the whole plant, tissue or cellular level. Burman *et al.* (2003) reported that increasing salinity progressively and significantly decreased seed yield. Incidental exposure to salt occurs either during the vegetative or reproductive or both phases, presenting an increasing threat to productivity. Low yield of grain under salinity has been reported due to the loss of photosynthetic capacity (Sultana *et al.*, 1999; Horton, 2000), decreased assimilates accumulation in the grain (Sultana *et al.*,1999; Asch *et al.*, 2000) and reduction of seed setting in the panicles (Khatun and Flowers, 1995). Sultana *et al.* (2001) reported that salinity had a slight effect on grain dry matter of rice at lower concentration and initial stages of growth, but the effect was aggravate by the high concentration and long duration of salinity.

Salt and drought stress have toxic effects on plants and result in diminished crop productivity. In the majority of plants these stresses provoke changes in gene expression leading to an increased synthesis of osmoprotectors and osmoregulators. Teixeira and Pereira (2007) characterized genes in potato for having low to moderate tolerance to salinity.

2.4 Efforts to reduce salinity problems

Plant responses to salinity are controlled by genes but an understanding of plant physiology is most likely to provide evidence concerning the perception of salinity stress by plants and effectors of salt stress tolerance. Plant survival depends on maintaining water potential, which is indispensable for expansion of growth and stomatal opening. The cellular tolerance processes to salt stress are those conserved in halophytic and glycophytic plants. The efforts should be focused on

(i) Sodium sequestration into the vacoule

(ii) Nutrient uptake

(iii) Osmoprotectant biosynthesis

(iv) Oxidative stress management

(v) Growth regulation

Among several factors that protect plant growth under salinity stress involving the above mentioned processes, plant hormones (phytohormones) have been identified to play a pivotal role. They have influence on plant metabolism under salinity stress through maintaining water potential, inducing uptake of nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) and synthesis of proteins and antioxidative enzymes.

A plant hormone is defined as a small organic molecule that elicits a physiological response at very low concentrations. The term plant hormone or phytohormone has been used for many years, but it has been suggested to refer these substances as plant growth regulators (PGRs), which have the following characteristics:

(i) Synthesized by plants and broadly distributed within the plant kingdom.

(ii) Show specific biological activity at very low concentration.

(iii) Play a fundamental role in regulating physiological phenomenon in a dose-dependent manner, which may change due to changes in the

sensitivity of the plant tissue during development or due to environment.

(iv) Different PGRs may interact, either synergistically or antagonistically to produce a particular effect.

PGRs generally display a very broad and complex action spectrum. In some cases, effects are observed immediately after the application of a PGR, while in other cases, it may take much longer to observe a change. It is presumed that the activities of existing enzymes or membrane properties are modified in fast reactions, while in the slowest reactions it is likely that gene expression (transcription or translation) is affected (Katekar, 1999).

In plants, five substances have been classically defined as plant hormones for many years. These are auxin, cytokinin, gibberellin, abscisic acid and ethylene. These are small molecules ranging from 28Da (ethylene) to 346Da (GA), all synthesized by the plants. In addition to the five classical hormones, some other substances are being studied that are currently considered to be PGRs. The other additional substances that gained the recognition as PGRs are polyamines, jasmonates, salicylic acid and brassinosteroids.

2.4.1 Salicylic acid: Can alleviate salinity effects?

Salicylic acid has been known to be present in some plant tissues for quite some time, but has only recently been recognized as a potential PGR. Salicylic acid is synthesized from the amino acid phenylalanine. The role of salicylic acid in the defence mechanisms against biotic and abiotic stresses has been well documented (Yalpani *et al.*, 1994; Szalai *et al.*, 2000). Salicylic acid has been found to promote flowering, stimulates plant pathogenesis protein production, enhances longevity of flowers, inhibits ethylene biosynthesis, and reverse the effects of ABA.

2.4.1.1 Salicylic acid biosynthesis

In plants, salicylic acid biosynthesis occurs *via* the shikimate–phenylpropanoid pathway (Zenk and Müller, 1964), where phenyl alanine is first converted to trans-cinnamic acid (t-CA) by phenylalanine ammonia lyase. Two pathways for the formation of salicylic acid have been reported in plants. t-CA is either hydroxylated to o-coumaric acid before oxidation of the side chain, or the t-CA side chain is shortened to benzoic acid (BA), which is in turn hydroxylated to salicylic acid (Sticher *et al.* 1997). Historically, the conversion of phenylalanine to cinnamic acid catalysed by phenylalanine ammonia lyase has been acknowledged as the rate-limiting step in the *de novo* biosynthesis of salicylic acid (Coquoz *et al.*1998).

2.4.2 Effects of salicylic acid on plants

Relatively little work has been done on the influence of this compound on plant metabolism. Salicylic acid plays an important role in flower induction, growth and development, ethylene biosynthesis, stomatal behaviour, and respiration (Raskin, 1992). It is important in disease resistance (Raskin, 1992; Klessig and Malamy, 1994) but the exact mode of the action of salicylic acid in this direction is not known.

Moharekar *et al.* (2003) reported that the total Chl (a+b) content decreased significantly in wheat with an increase with the SA concentration. However, in moong, Chl content was lower in control plants than in salicylic acid treated ones but decreased significantly with the increase in salicylic acid concentration. A reduction in chlorophyll content in barley and moong leaves following the application of SA was found by Pancheva *et al.* (1996) and Anandhi and Ramanujam (1997).

Moharekar *et al.* (2003) reported that Chl a/b ratio decreased significantly with an increase in SA concentration in wheat. However, in moong it remained constant. In contrast to Chl, the content of total Car increased significantly with an increase in SA concentration in both the crops.

increase in SA concentration stimulated Car accumulation in wheat and moong. They also reported that the size of xanthophyll pool increased significantly with the increase in SA concentration. Under stress, zeaxanthin and possibly antheraxanthin have been found responsible for the quenching of excess excitation energy (Gilmore and Yamamoto, 1993). The size of xanthophylls increases under stress, (Demmig-Adams *et al.*, 1989, 1995; Logan *et al.*, 1996).

SA is most important systemic signal molecule. Several attempts have been made to induce resistance by increasing SA content of plants. Exogenous application of SA has induced resistance in some plants (Conrath *et al.*, 1995; Lawton *et al.*, 1996; Amaresh-Chandra *et al.*, 2001; Guleria *et al.*, 2001; Negi and Prasad, 2001; Vasudha *et al.*, 2001).

Keshamma *et al.* (2004) reported the effect of SA on germination of chickpea seeds. Seeds treated with water and 1mM SA concentration started to germinate on 1d of sowing while seeds treated with SA at 2mM, 3mM, 4mM concentration started to germinate on 2, 3, 4d, respectively. However, at 5mM concentrations no seeds were germinated even after 5d of sowing. An exogenous supply of SA affects seedling growth/seed germination. Higher concentrations of SA decreased germination process in soybean seeds (Negi and Prasad, 2001). Convincing data are available concerning the SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova, 1997), and water deficit (Bezrukova *et al.*, 2001) of tomato and bean plants to low and high temperature (Senaratna *et al.*, 2000), as well as the injurious action of heavy metals on rice plants (Mishra and Choudhuri, 1999).

The important role of SA in protection is probably played by its ability to induce expression of genes coding not only for proteins but also the extensin gene in *Arabidopsis* plants (Merkouropoulos *et al.*, 1999). There are data about

SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova *et al.*, 1999) and accumulation of wheat lectins (Shakirova and Bezrukova, 1997), fast activation of 48 kDa protein kinase in suspension cell culture of tobacco at osmotic stress (Mikolajczyk *et al.*, 2000). This suggested the involvement of SA in realization of different anti stress programs. However, the way of signal regulation of plant resistance to unfavorable factors of environment induced by SA is still not clear.

Pre-sowing treatment of wheat seeds with SA contributed to the increase in the resistance of plants to stress factors of environment and ABA served as a mediator in the manifestation of the protective action of SA. SA treatment induced a sharp accumulation of ABA, which in turn is an inducer of a wide spectrum of anti stress reactions in plants. Maintaining a high level of ABA in SA treated plants under stress contributed to the protective reactions aimed to decrease its injurious effects on growth and acceleration of growth resumption. Several studies have supported a major role of SA in modulating the plant response to several abiotic stresses, such as ultraviolet light, drought, salt, chilling and heat (Yalpani *et al.*, 1994; Dat *et al.*, 1998a, b; Janda *et al.*, 1999; Mishra and Choudhuri, 1999; Senaratna *et al.*, 2000). In maize plants, pretreatment with SA or aspirin caused a decrease in net photosynthesis under normal growth conditions (Janda *et al.*, 1999, 2000).

Popova *et al.* (2003) reported that treatment of barley seedlings with SA in the dark followed by 6h light exposure did not cause wilting or irreversible damage to photosynthesis. Concentrations of SA are very relevant for physiological studies. They did not cause visible damage symptoms after long-term treatment, but provided well-reproducible and reversible effects on photosynthesis, growth and biochemistry of barley plants. Treatment of barley seedlings with SA caused an inhibition in the net photosynthetic rate. Dark-treated barley seedlings with SA did not show loss in chlorophyll content. Pretreatment of plants with SA before paraquat application caused a protection

against paraquat-induced chlorophyll losses. No significant changes in the protein levels were observed in SA and dark-treated seedlings. Pretreatment with SA before paraquat prevented the protein loss RuBPC (ribulose-1,5-biphosphate carboxylase) activity was almost unaffected when plants were treated with SA (Popova *et al.*, 2003). Pretreatment with SA before application of paraquat had no effect on the enzyme activity.

Several studies carried out under laboratory or field conditions strongly suggest that SA and other salicylates play an important role in many biological responses in plants. The effect of these substances on the physiology of the plants is variable, promoting some processes and inhibiting others (Raskin, 1992). Significant reductions in transpiration and stomatal aperture were obtained, but SA has also been reported to reverse the stomatal closure induced by ABA (Rai *et al.*, 1986). Exogenous applications of SA to different species of crops have been shown to elicit effects on yield and yield components. An increase in the number of pods and yield has been found in mungbean (Singh and Kaur, 1980), *P. vulgaris* (Rendon, 1983; Lang, 1986) and wheat (Lopez, 1989). Other effects of SA and its regulatory role in plant physiology included inhibiting ethylene biosynthesis, interfering with membrane depolarisation, blocking wound responses, and an increase in net photosynthetic rate and chlorophyll content in soybeans (Glass and Dunlop, 1974; Leslie and Romani, 1988; Zhao *et al.*, 1995). It has also been recognized that SA is required in the signal transduction chain for inducing systemic acquired resistance (Metraux *et al.*, 1990; Gaffney *et al.*, 1993; Vernooij *et al.*, 1994). Zhao *et al.* (1995) reported an increase in net photosynthetic rate that they ascribed to an enhancement of leaf enzyme activity by SA. Gutiérrez-Coronado *et al.* (1998) reported that, in soybean, shoot growth was increased with the concentrations of SA. The concentrations of SA significantly increased root length. Singh (1993) found that SA stimulated root formation in young shoots of ornamental

plants and Li and Li (1995) reported the formation of adventitious roots on hypocotyl cuttings of mungbeans.

Exogenous application of SA enhanced the drought and salt stress resistance of plants (Senaratna *et al.*, 2000; Tari *et al.*, 2002), but the results were contradictory and depended on the developmental phase of plants (Borsani *et al.*, 2001) or on the experimental conditions (Nemeth *et al.*, 2002).

Szepesi *et al.* (2005) reported that the 10^{-7} M SA pretreatment in tomato decreased the osmotic stress-induced reduction in relative water content, but this alleviating effect was not so pronounced at 10^{-4} M SA concentration. A small increase was observed in the water and osmotic potential of SA pretreated samples, but in case of the pressure potential the changes were higher. Under the influence of salt stress the osmotic potential greatly decreased and the SA pretreatments moderated it at both 10^{-7} M and 10^{-4} M concentrations. SA pretreatments reduced K^+ contents of leaves under salt and non-ionic osmotic stress. Compared to the NaCl-treated plants, SA decreased the Na^+/K^+ ratio in the roots and increased it significantly in the leaves. SA improved the photosynthetic performance of plants under stress conditions (Ananieva *et al.*, 2002), and chlorophyll a fluorescence gave insight into the ability of plant to tolerate environmental stresses. Szepesi *et al.* (2005) reported that at low photosynthetic light intensity (165μmol m^{-2} s^{-1}) the effective quantum yield was only slightly affected in NaCl-treated tomato samples, but it was significantly reduced under non-ionic osmotic stress. This was partially overcome if the plants were pretreated with SA. SA pretreatment might improve the gross rate of carbon assimilation during osmotic stress.

Pan *et al.* (2006) reported that SA application reduced leaf injury in pea (*Pisum sativum*) caused by heat stress and induced the synthesis of heat shock proteins (Hsp70 and Hsp17.6). Further, membrane lipid peroxidation caused by the heat stress was found to decrease, suggesting that plant's thermo-tolerance developed as a result of SA application. A rapid transient increase of

endogenous free SA and a subsequent enrichment in Hsp70 were both elevated by heat acclimation. Gunes *et al.* (2007) reported that exogenously applied SA increased plant growth of maize significantly both in saline and non-saline conditions. As a consequence of salinity stress, lipid peroxidation, measured in terms of malondialdehyde content and membrane permeability decreased by SA. UV-absorbing substances (UVAS) and H_2O_2 concentration were increased by increasing levels of SA. SA also strongly inhibited Na^+ and Cl^- accumulation, but stimulated N, Mg, Fe, Mn and Cu concentrations of salt stressed maize plants. It was concluded that SA could be used as a potential growth regulator to improve plant salinity stress resistance.

2.4.3 Salicylic acid, oxidative stress and components of antioxidant defence

SA has been reported to influence the activities of antioxidative enzymes differentially. SA inhibited the activities of catalase and ascorbate peroxidase and increased the content of H_2O_2 (Chen *et al.*, 1993; Durner and Klessig, 1995; Rao *et al.*, 1997; Kawano and Muto, 2000; Luo *et al.*, 2001). Moharekar *et al.* (2003) suggested that an increase in SA concentration might induce oxidative stress in wheat and moong but the degree of oxidative stress was different in different plant species. Catalase activity from cucumber, tomato, *Arabidopsis* and tobacco has been found substantially inhibited by SA, whereas those from maize and rice were found insensitive (Sanchez-Casas and Klessig, 1994). In contrast, Keshamma *et al.* (2004) found that catalase activity in roots of chickpea was not inhibited by SA *in vitro*. At 200mM NaCl concentration only 30% inhibition was observed. However, when seeds were soaked at different time intervals in 1mM SA, there was a complete inhibition of catalase activity. The treatment of SA+ spermine enhanced total guaicol peroxidase activity by about 20 fold and 100 fold in seeds and roots, respectively compared to control.

Stimulation or inhibition in the activities of perxoidase, phenylalanine ammonia lyase by SA has been reported to be concentration dependent (Jain

and Srivastava, 1981a, b). It has been reported that SA increased peroxidase, phenylalanine ammonia lyase activities by inducing the synthesis of enzymes as well as by some kind of direct modulation of the enzyme molecules (Singh and Srivastava, 1987). Kauss *et al.* (1992) also observed increase in peroxidase activity in parsley cells in response to 2,4-dichloro-isonicotinic acid and SA spray. The increase in peroxidase activity after SA spray has also been reported in salt-exposed rice seedlings (Cai and Zheng, 1997).

Shim *et al.* (2003) reported a significant and dose-dependent increase in SA content in the NaCl-treated leaves of rice seedlings. They negatively correlated this increase in SA content with catalase activity and concluded that the formation of SA could be induced by salt stress.

Exogenous SA treatment could induce an increase in H_2O_2 levels in plant tissues. In maize plants, pretreatment with SA or aspirin activated some antioxidant enzymes (peroxidase and glutathione reductase), which in turn increased chilling tolerance (Janda *et al.*, 1999, 2000).

Kang *et al.* (2003) reported that banana treated with 0.5mM SA at 30/22°C for 1d did not change superoxide dismutase activity. A chilling stress of 3d at 5°C quickly reduced superoxide dismutase activity both in control and SA pretreated plants. Superoxide dismutase activity in leaves of SA pretreated seedling was significantly higher than in the control plants. At 30/22°C, SA treatment for 1d markedly inhibited catalase and ascorbate peroxidase activities. A chilling stress of 3d at 5°C caused a rapid decrease of catalase and peroxidase activities in leaves of control plants, while it significantly induced an increase in the activities of catalase and ascorbate peroxidase in SA pretreated leaves. Changes in the enzyme activities such as superoxide dismutase and H_2O_2 degrading enzymes such as catalase, ascorbate peroxidase and peroxidase induced by SA treatment resulted in the difference of H_2O_2 levels. In *Arabidopsis,* SA was found necessary for the induction of antioxidant defenses and maintaining the redox state of glutathione pool (Sharma *et al.*,

1996). Thus, SA has been shown to be essential for the plant protection against the oxidative stress generated by ozone (Rao and Davis, 1999).

Szepesi *et al.* (2005) reported that SA pretreatment decreased catalase activity in the roots and leaves of tomato, but the activity of other enzymes associated with the antioxidative defence, superoxide dismutase, peroxidase, ascorbate peroxidase and glutathione reductase exhibited different changes at 10^{-7}M SA or 10^{-4}M SA. The activity of these enzymes decreased compared to the control in the leaves of tomato plants at 10^{-7}M SA pretreatment, while at 10^{-4}M concentration their activity was enhanced. Salt tolerance induced by 10^{-4}M SA was associated with the activation of the oxidative defence mechanisms and with the accumulation of osmolytes. Wang and Li (2006) noticed that exogenous SA pretreatment decreased thiobarbituric acid reactive substances and relative electrolyte leakage in grape leaves under heat or cold stress. Exogenous SA pretreatment enabled the grape leaves to maintain relatively higher activities of ascorbate peroxidase, glutathione reductase, monodehydroascorbate and the redox ratio in the ascrobate glutathione pool under normal temperature and under heat or cold stress. Cytosolic Ca^{2+} in SA-treated mesophyll cells was greater than that in controls at the normal temperature. SA treated cells maintained Ca^{2+} homeostasis under cold or heat stress and increased tolerance.

2.5. Critical appraisal of the review of literature

The literature reviewed above includes studies on the physiological analysis of various growth, photosynthetic traits, biochemical traits, antioxidant studies and yield of crop plants under salt stress. It appears that there are few reports concerning the effect of salicylic acid on the physiological processes and productivity of important crops viz. mungbean and mustard. In fact, the works reported on various crop plants are invariably aimed at establishing the effects of salicylic acid on biochemical and physiological characteristics and associated changes in the tolerance. However, our understanding on the

response of SA in enhancing/strengthening tolerance of plants and alleviating the effects of salt stress requires more experimentation and studies.

The influence of salicylic acid on the alleviation of salt stress in mungbean and mustard has not been studied. Keeping in view the attention focused on SA in recent years as phytohormone and its effect on various aspects of physiological, biochemical and metabolic characteristics, the present investigation was undertaken to explore the possibility of ameliorating salt stress effects on physiological and biochemical traits, antioxidant system and productivity of mungbean and mustard.

MATERIALS AND METHODS

Contents

MATERIALS AND METHODS

MATERIALS AND METHODS

The chapter deals with the description of materials used for the study and methods adopted for the experimentation and determination of various traits during the course of the investigation.

3.1 Experimental Materials

Experiments 1 and 3 were conducted on mungbean and Experiments 2 and 4 on mustard.

Seeds of mungbean (*Vigna radiata* L. Wilczek) cvs. T44, Pusa Vishal, Tram and PDM54 and Indian mustard (*Brassica juncea* L. Czern & Coss.) cvs. Alankar, Pusa Bold, Sakha and PBM16 were obtained from the Regional Research Centre, Kanpur, India.

3.1.1 Botanical Description of Mungbean

3.1.1.1 Nomenclature

A large number of types are grown in India. They are often grouped under distinct varieties or sub-species. The most important among them are:

I. var. *radiata* with dark-green foliage with spreading pods and green seeds.

II. var. *aurea* (Roxb.) Prain syn. *Phaseolus aureus* Roxb., with pale foliage, reflexed pods and yellow seeds.

III. var. *grandis* Prain having medium-green foliage, large spreading pods and black seeds.

IV. var. *brunea* Bose having medium-green foliage, spreading pods and brown seeds.

V. var. *sublobata* (Roxb.) Verdcourt syn. *Phaseolus trinervius* Wight & Arn., *P. sublobatus* Roxb. which is considered to be the wild mung and

var. *globa* (Roxb.) verdcourt syn. *Phaseolus glaber* Roxb. *Phaseolus mungo* var. *glaber* (Bose, 1932).

3.1.1.2 Botanical description

Mungbean belongs to the family Leguminosae and sub-family Paplionaceae. It is commonly known as green gram and golden gram. It is an erect or sub-erect annual, cultivated almost throughout the India. Stem is 45-120cm high, with a slight tendency of twining in its upper branches; leaves are trifoliate; leaflets entire, rarely trilobed, ovate in outline; flowers are yellow or yellowish green and crowded in clusters of 10-25 in number on long pedicels; pods are 5-10cm long, thin, cylindrical; seeds more or less globular, mostly green in colour, but sometimes marbled black and green, yellow, brown purple brown, the surface exhibits many fine, wavy ridges, hilum flat, covered with a white rough layer.

3.1.1.2.1 Genomic relationships of *Vigna radiata*

Hybrids with high fertility have been reported in *V. radiata* × *V. radiata* var. sublobata and its reciprocal (Chavan *et al.*, 1965; Biswas and Dana, 1975). The meiosis is regular with 11 bivalents in most of the cells in the hybrid of *V. radiata* × *V. radiata* var. sublobata (Biswas and Dana, 1975).

Partial fertile seed hybrids are obtained from the crosses of *V. radiata* with *V. mungo* (Dana, 1966), *V. mungo* with *V. radiata* var. sublobata (Chavan *et al.*, 1965; Biswas and Dana, 1975). Meiosis is regular with 10-11 bivalents in many cells in the hybrids from the crosses of *V. radiata* with *V. mungo*. Therefore, Dana (1966) suggested that all the three species, *V. mungo*, *V. radiata* and *V. radiata* var. sublobata can be represented with AA genome.

3.1.2 Botanical Description of Mustard

3.1.2.1 Nomenclature

The oleiferous *Brassica* grown in India are divided into four groups:

1. Brown mustard: Commonly known as rai, raya or laha (*Brassica juncea* L. Czern & Coss.)
2. Sarson

   a. Yellow sarson: *Brassica campestris* L. var. Sarson Prain

b. Brown sarson: *Brassica campestris* L. var. Dichotoma Watt

3. Toria: lahi or maghi lahi *Brassica campestris* L. var. Toria Duth
4. Taramira or Tara (*Eruca sativa* Mill.)

In addition, there are two other species, namely *Brassica nigra* Koch. (Banarasi rai) and *Brassica juncea* var. Rugosa (Pahadi rai). These do not fall under any of the four groups. These are, moreover, grown to a limited extent. Mustard (*Brassica juncea* L. Czern & Coss.) is the dominant species grown in India (Prakash, 1980).

3.1.2.2 Botanical description

Rape and mustard include annual herbs. Roots, in general, are long and tapering. Toria is more or less a surface feeder but Brown sarson bears long roots with limited lateral spread enabling its successful cultivation under drier conditions. The height of the stem varies from 45cm (in some varieties of Toria) to 190cm (in Yellow sarson). In Toria and Brown sarson, the branches arise at an angle of 30° to 40°. In Yellow sarson, the branches arise laterally at an angle of about 10° to 20° and give the plant a narrow and pyramidal shape. The inflorescence is a corymbose raceme. In the case of Yellow sarson, the four petals are spread apart, whereas in Brown sarson and Toria, the petals overlap or may be placed apart, depending upon the cultivar. The flowers bear a hypogynous ovary. In Brown sarson and Toria, the ovary is bicarpellary, whereas in Yellow sarson, it may also be tri-or tetra-carpellary. The fruit is sliliqua. The pods are two-valved, three-valved or four-valved, depending upon the number of carpels in the ovary. The flowers begin to open from 8.00h and continue up to 12 noon.

3.1.2.2.1 Genomic relationships of *Brassica juncea*

The modern understanding of genomic relationships among the *Brassica* species and cytological evidence show that *Brassica napa* (n=10, A), *Brassica nigra* (n=8, B) and *Brassica oleraceae* (n=9, C) are primary species, and

Brassica juncea (n=18, AB) is an amphidiploid resulting from a cross between *Brassica napa* and *Brassica nigra* (Morinaga, 1934).

3.2 Climatic Conditions of Aligarh

Aligarh is situated at 27°52'N, 78°51'E and 187.4m altitude above the sea level in the mid of Doab, the land between the Ganga and Yamuna rivers at a distance of 130km southeast of Delhi on the Delhi-Howrah rail route.

Aligarh experiences semi-arid and subtropical climate, with hot dry summer and cold winters. The summer season extends from April to June. In this season maximum temperature sometimes reaches to 46°C in the month of June.

The winter varies from the middle of October till the end of March. The temperature in December and January reaches as low as 15°C and 13°C, and lowest recorded for any single day is 2°C and 0.5°C, respectively. The mean annual rainfall is about 847.3mm. More than 85% of the total downpour is delivered during a short span of four months from June to September. The remaining rain showers are received during winter.

3.3 Experimentation

The experiments were conducted in sand in earthen pots during the summer season of 2003 and 2004 on mungbean (*Vigna radiata* L. Wilczek) and the winter season of 2003 to 2005 on mustard (*Brassica juncea* L. Czern & Coss.) in the green house of the Department of Botany, Aligarh Muslim University, Aligarh, India. The experimental period varied from April to June for *Vigna radiata* and October to March for *Brassica juncea*.

3.3.1 Sand Culture

3.3.1.1 Purification of sand

Before the beginning of each experiment, sand purification was done adopting the method of Hewitt (1966). First of all the coarse sand was washed thoroughly with tap water, then treated with 18% HCl for 24h followed by

washing with deionized water and drying the sand completely. The acid-washed sand was used for filling 15cm diameter earthen pots.

3.3.2 Preparation of Nutrient Solution

Hoagland nutrient solution for plant culture

Solution 1 contained following salts (g l^{-1})

KH_2PO_4	:	0.136
KNO_3	:	1.02
$Ca(NO_3)_2$	:	0.492
$MgSO_4.7H_2O$	:	0.49

Solution 'A' contained following salts (mg l^{-1})

H_3BO_3	:	2.86
$MnCl_2.4H_2O$	:	1.81
$ZnSO_4.7H_2O$	:	0.22
$CuSo_4.5H_2O$	:	0.08
$H_2MoO_4.H_2O$	:	0.09

Solution 'B'

26.1g EDTA was dissolved in 268ml of 1N KOH. To this, 24.9g $FeSO_4.7H_2O$ was added and diluted to one litre. The solution was aerated overnight to produce stable ferric complex. The pH of the solution was 5.5.

One ml of solution 'A' was mixed into one litre of Solution 1 and to it 1ml of Solution 'B' was added. The pH of the solution was adjusted to 6 with 0.1N H_2SO_4.

3.3.3 Experimental Lay-out

The treatments in each experiment were arranged in a randomized block design. Each determination was repeated three times. Each pot had two plants. In Experiment 1, three pots were used for the determination of growth, another three pots for the measurement of photosynthesis at each sampling time. For yield also three pots were used. Thus, in all fifteen pots were maintained for each treatment. In Experiment 2, similar distribution of pots was made, but due

to three sampling stages and harvest, the total number of pots per treatment was twenty one. In Experiments 3 and 4, the distribution of pots for determining various characteristics was same. Biochemical characteristics were determined in plants selected for the growth determination. Similarly, the activities of antioxidative enzymes were measured in plants leaves used for photosynthesis measurement.

Calender of various operations in each experiment is given in Table 1 and shows time schedule for experimentation, treatment and the data collection. Seeds of mungbean or mustard were sown in 15cm-diameter pots containing purified sand. After the seedling establishment, two plants per pot were maintained. Prior to sowing, 900ml Hoagland solution was given to all pots. In order to check the aphid contagion, if any, insecticidal spray of Dimecron-100 was done with a hand spray.

3.3.3.1 Experiments 1 and 2

Experiment 1 (mungbean) was conducted in the summer season of 2003. Seeds were sown on 5th April 2003 and the crop was harvested on 5th June 2003. Experiment 2 (mustard) was conducted in the winter season of 2003-2004. Seeds were sown on 15th October 2003 and the crop was harvested on 13th Feberuary 2004. The aim of these experiments was to assess the effects of 0, 50 and 100mM NaCl on growth, photosynthetic and yield characteristics, and select tolerant and non-tolerant cultivars of mungbean and mustard. The different concentrations of NaCl were given along with 300ml Hoagland solution every day in the morning, whereas only Hoagland solution was given in the evening in mungbean. Mustard crop was fed with 300ml Hoagland solution containing NaCl treatments once a day in the morning. Flushing was done once a week to remove excess NaCl, if any. Sampling was done at 20, 40 and 60DAS (maturity) in mungbean and 30, 60, 90 and 120DAS (maturity) in mustard. Scheme of the treatments for the experiments is given in Table 2.

Calendar of Operations

Table 1: Different dates showing experimentation of mungbean and mustard

Experiment	1 (2003)	2 (2003-2004)	3 (2004)	4 (2004-2005)
1. Preparation of sand for experiment	01-04-2003	10-10-2003	03-04-2004	15-10-2004
2. Sowing	05-04-2003	15-10-2003	07-04-2004	20-10-2004
3. Treatments				
A. NaCl	09-04-2003	19-10-2003	11-04-2004	24-10-2004
B. SA			23-04-2004	05-11-2004
4. Sampling				
A. 1st	26-04-2003	15-11-2003	28-04-2004	20-11-2004
B. 2nd	16-05-2003	15-12-2003	18-05-2004	20-12-2004
C. 3rd		14-01-2004		19-01-2005
5. Harvesting	05-06-2003	13-02-2004	07-06-2004	18-02-2005

Table 2: An outline of Experiment 1 and Experiment 2

Experiment 1

Cultivars	NaCl (mM)		
	0	50	100
T44	+	+	+
Pusa Vishal	+	+	+
Tram	+	+	+
PDM54	+	+	+

Crop : Mungbean (*Vigna radiata* L. Wilczek)

Experiment 2

Cultivars	NaCl (mM)		
	0	50	100
Alankar	+	+	+
Pusa Bold	+	+	+
Sakha	+	+	+
PDM16	+	+	+

Crop : Mustard (*Brassica juncea* L. Czern & Coss.)

3.3.3.2 Experiments 3 and 4

The experiments were conducted during the summer and winter season of 2004-2005. In mungbean sowing was done on 7th April 2004 and harvested on 7th June 2004. The sowing of mustard seeds was done on 20th October 2004 and the crop was harvested on 18th Feberuary 2005. These experiments were conducted on the basis of the observations of Experiments 1 and 2. The aim of these experiments was to assess the influence of salicylic acid application on foliage of plants on plant development and alleviation of salinity stress. The SA was applied at a concentration of 0.0, 0.1, 0.5 and 1.0mM at 15DAS. The plants were grown with 0 or 50mM NaCl. The schedule of NaCl treatments was same as for Experiments 1 and 2. The treatment of 100mM NaCl was discarded as this proved lethal at later growth stages on both the crops (Experiments 1 and 2). The plant development was studied in terms of growth, photosynthetic and biochemical characteristics, activities of antioxidative enzymes and yield characteristics. The treatments were arranged in a complete randomized block design with three replicates. The timing of sampling in the two crops was same as for earlier experiments. The scheme of treatments is given in Table 3.

3.3.4 Plant Sampling

3.3.4.1 Experiments 1 and 2

Data on growth and photosynthetic characteristics were recorded at 20 and 40DAS and yield characteristics at 60DAS (maturity) in mungbean. In mustard, data on growth and photosynthetic characteristics were collected at 30, 60 and 90DAS and for yield characteristics at 120DAS (maturity).

3.3.4.2 Experiments 3 and 4

In these two experiments growth, photosynthetic and biochemical characteristics were measured at 20 and 40DAS in mungbean and 30, 60 and 90DAS in mustard. The yield characteristics of the two crops were recorded at 60 and 120DAS, respectively. The activities of antioxidative enzymes were

Table 3: An outline of Experiment 3 and Experiment 4

Experiment 3

Cultivars	Pusa Vishal (Salinity tolerant)	Tram (Salinity non-tolerant)
NaCl (0mM)		
SA (mM)		
0.0	+	+
0.1	+	+
0.5	+	+
1.0	+	+
NaCl (50mM)		
SA (mM)		
0.0	+	+
0.1	+	+
0.5	+	+
1.0	+	+

Crop: Mungbean (*Vigna radiata* L. Wilczek)

Experiment 4

Cultivars	Alankar (Salinity tolerant)	PBM16 (Salinity non-tolerant)
NaCl (0mM)		
SA (mM)		
0.0	+	+
0.1	+	+
0.5	+	+
1.0	+	+
NaCl (50mM)		
SA (mM)		
0.0	+	+
0.1	+	+
0.5	+	+
1.0	+	+

Crop: Mustard (*Brassica juncea* L. Czern & Coss.)

also measured in these two experiments at 20DAS in mungbean and 30DAS in mustard.

3.3.5 Chemicals

Reagents and chemicals used in the study were analytical grade obtained from Sigma or S.D. Fine Chemicals.

3.3.6 Observations

3.3.6.1 Growth characteristics

Following growth characteristics were studied:

1. Root length per plant
2. Root fresh mass per plant
3. Root dry mass per plant
4. Leaf fresh mass per plant
5. Leaf dry mass per plant
6. Leaf area per plant
7. Plant dry mass per plant

Plants were uprooted carefully from the pots, washed to remove dust, if any and root length was measured on a meter scale. Fresh mass of root and leaf was recorded. The plants of which fresh mass was taken were dried separately in a hot-air oven at 80°C till constant weight. The dried material was weighed on an electrical balance and the weight was recorded as dry mass of different plant parts as well as whole plant dry mass. Leaf area of functional plant leaves was determined with a LA21 leaf area meter (Systronics, Ahmedabad, India).

3.3.6.2 Photosynthetic characteristics

Following photosynthetic characteristics were observed:

1. Carbonic anhydarase activity
2. Net photosynthetic rate
3. Stomatal conductance (given for Experiment 3 and 4)
4. Intercellular CO_2 concentration (given for Experiment 3 and 4)
5. Chlorophyll and carotenoid content (given for Experiment 3 and 4)

3.3.6.2.1 Assay of carbonic anhydrase activity

Carbonic anhydrase (CA) facilitates the supply of CO_2 to the carboxylation sites. It catalyzes the reversible hydration of carbon dioxide (Raven, 1995; Khan *et al.*, 2004).

$$H_2O + CO_2 \underset{}{\overset{CA}{\rightleftharpoons}} H^+ + HCO_3^-$$

3.3.6.2.1.1 Estimation

Carbonic anhydrase activity was measured by adopting the method of Dwivedi and Randhava (1974). Leaves used for photosynthesis measurements were used for the enzyme assay. Leaves were cut into small pieces (2-3mm length) in 10ml 0.2M cystein in *Petri dish* at 0-4°C. The solution adhering to the leaf surface was removed with the help of blotting paper followed by the immediate transfer of leaves to test tube having 4ml of phosphate buffer (pH 6.8). To this, 4ml of 0.2M sodium bicarbonate in 0.02M sodium hydroxide solution and 0.2ml 0.002% bromothymol blue indicator were added to the tubes. The tubes were kept at 4°C for 20min.

CO_2 librated during catalytic action of the enzyme on sodium bicarbonate was estimated by titrating the reaction mixture against 0.05N hydrochloric acid, using methyl red as an indicator. The control reaction mixture was also titrated against 0.05N hydrochloric acid. The difference of the sample and the control readings was noted for the calculation of the enzyme activity.

3.3.6.2.2 Net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration

Net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration were measured in fully expanded uppermost leaves of plants in each treatment using Infra Red Gas Analyzer (LiCOR-6200, Portable Photosynthesis System, Nebraska, USA). The measurements were done on sunny days. Atmospheric CO_2 concentration during the measurement was 360±2μmol mol^{-1}. The ambient temperature for mungbean was 30±2°C and

23±1°C for mustard and photosynthetically active radiation was 950±25 and 800±28μmol $m^{-2}s^{-1}$ for mungbean and mustard, respectively.

3.3.6.2.3 Chlorophyll and carotenoid contents

Chlorophyll and carotenoid were extracted using the method of Hiscox and Israelstam (1979) by using dimethyl sulphoxide (DMSO) as an extraction medium, and estimated by the method of Arnon (1949).

3.3.6.2.3.1 Extraction

Fresh leaves (100mg) were cut into small pieces and collected in test tubes containing 7.0ml of dimethyl sulphoxide. The test tubes were covered with black paper and incubated at 45°C for 40min for the extraction. The reaction mixture was transferred to a graduated tube and the final volume was made to 10.0ml with DMSO.

3.3.6.2.3.2 Estimation

Extract measuring 3.0ml was transferred to a cuvette and the absorbance was read at 645 and 663nm for chlorophyll and at 480 and 510nm for carotenoid on SL164 UV-VIS Spectrophotometer (Elico, Hyderabad, India).

3.3.6.2.3.3 Calculation for chlorophyll and carotenoid contents

Total chlorophyll content was calculated according to the equation given by Arnon (1949).

$$\text{Total Chlorophyll (mg g}^{-1}\text{ leaf fresh mass)} = [20.2\,(OD_{645}) + 8.02\,(OD_{663})] \times \frac{V}{1000 \times W}$$

Where, V = volume of the extract

W = mass of the leaf tissue taken

$$\text{Carotenoid (mg g}^{-1}\text{ leaf fresh mass)} = \frac{7.6(OD_{480}) - 1.49(OD_{510})}{d \times 1000 \times W} \times V$$

OD = optical density at the given wave lengths viz. 645, 663, 480 and 510nm

3.3.6.3 Biochemical characteristics

Biochemical characteristics were determined in Experiment 3 and 4.

Following biochemical characteristics were determined in dried material, collected at different sampling times.

1. Leaf Na concentration
2. Leaf Cl concentration
3. Leaf Ca concentration
4. Leaf N concentration
5. Leaf P concentration
6. Leaf K concentration
7. Leaf protein concentration

3.3.6.3.1 Digestion of leaf sample for the estimation of Na, Cl and Ca concentrations

50mg of oven dried leaf material was taken in a 50ml volumetric flask. To this, 2ml concentrated nitric acid was added and it was heated on an electric hot plate till the appearance of brown effervescence. At the stop of effervescence, Tri acid mixture (TAM) solution was added till a clear solution was obtained. TAM is a mixture of nitric acid, sulphuric acid and perchloric acid mixed in the ratio of 10:5:4. The material was then allowed to dry on hot plate. After drying, 50ml of DDW was added, shaken and transferred into another 50ml volumetric flask with three washings with DDW. The final volume was made up to the mark with DDW.

3.3.6.3.1.1 Estimation of Na

Flame photometer (2273, Khera, New Delhi, India) was used to read the sodium concentration in the digested samples using sodium filter.

3.3.6.3.1.1.1 Preparation of standard curve

Standard curve was prepared by taking known concentrations of sodium. 5.845g of NaCl was dissolved in DDW and the volume was made to 1 litre, that gave 100 milliequivalents per litre of Na. Different dilutions of 5, 20, 30, 40 and 50meq Na was prepared from the stock solution. The concentrations of

Na in the unknown sample were read from the graph plotted with the readings of flame photometer and dilutions.

3.3.6.3.1.2 Estimation of Cl

50ml of digested leaf sample was taken in a flask and 2ml of 5% K_2CrO_4 indicator was added. It was titrated against 0.02N silver nitrate solution and calculated as follows:

$$\text{Chloride (mg } l^{-1}) = \frac{(A - B)\,N \text{ of } AgNO_3 \times 1000 \times 35.5}{\text{ml sample}}$$

Where A = ml titration for sample

B = ml titration for blank

3.3.6.3.1.3 Estimation of Ca

The calcium in digested samples was estimated with the help of the flame photometer.

3.3.6.3.1.3.1 Preparation of standard curve

2.497g $CaCO_3$ in 15ml of concentrated HCl was dissolved and the volume was made to 1 litre with DDW. Different dilutions of 0, 100, 200, 300, 400 and 500ppm calcium were prepared from 1000ppm calcium solution. The readings were directly read on the flame photometer. A standard curve, taking known dilutions of standard $CaCO_3$ solutions, was plotted. The reading of each sample was compared with this calibration curve and calcium in samples was calculated on dry mass basis.

3.3.6.3.2 Digestion of plant sample for the estimation of N, P and K concentrations

Oven dried leaf sample was ground in a mortar and pestle to prepare fine powder. Powder weighing 100mg was transferred to a 50ml Kjeldahl flask to which 2ml sulphuric acid was added. The contents of the flask were heated on a temperature-controlled assembly for 2h to allow complete reduction of nitrates in the plant material by the organic matter itself. As a result, the contents of the flask turned black. After cooling the flask for about 15min,

0.5ml of 30% H_2O_2 was added drop by drop and the solution was heated again until the colour turned to light yellow. After further cooling for about 30min, additional 3-4 drops of H_2O_2 were added followed by heating for another 15min. The process was repeated till the light yellow colour turned colourless. The digested material was transferred to a 100ml volumetric flask with three washings with DDW. The volume of the flask was maintained up to the mark.

3.3.6.3.2.1 Estimation of N

Leaf nitrogen concentration was estimated by the Kjeldahl digestion method as described by Lindner (1944).

A 10ml aliquot of the digested material was taken in 50ml volumetric flask. To the flask, 2ml of 2.5N NaOH and 1ml of 10% sodium silicate solution were added to neutralize the excess of acid and prevent turbidity. The volume of the solution was made up to the mark with DDW. In a 10ml graduated test tube 5ml of the solution was taken and 0.5ml of Nessler's reagent was added. The final volume was maintained with DDW. The content of the tube was allowed to stand for 5min for maximum colour development. The intensity of the solution was read spectrophotometrically at 525nm.

3.3.6.3.2.1.1 Preparation of standard curve

50mg ammonium sulphate was dissolved in DDW to get 1 litre solution. From this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0ml solution was taken in ten different test tubes. The solution in each test tube was diluted to 5ml with DDW. In each test tube 0.5ml Nessler's reagent was added. After 5min, the intensity of the colour was read at 525nm. A blank was run simultaneously with each set of determination.

Standard curve was plotted using different concentrations of ammonium sulphate solution versus optical density and with the help of the standard curve, the amount of nitrogen present in the sample was determined on dry mass basis.

3.3.6.3.2.2 Estimation of P

The method of Fiske and Subba Row (1925) was adopted for the estimation of phosphorus. A 5ml aliquot was taken in 10ml graduated test tube and 1ml of 2.5% molybdic acid reagent was carefully added followed by the addition of 0.4ml of 1-amino-2-naphthol-4-sulphonic acid. The addition of this solution turned the colour of the contents blue. Volume was made up to 10ml. The solution was shaken for 5min for maximum colour development and subsequently transferred to a colorimetric tube. The intensity of the colour was read at 620nm. A blank was run simultaneously.

3.3.6.3.2.2.1 Preparation of standard curve

351mg monobasic dihydrogen orthophosphate was dissolved in sufficient DDW to which 10ml of 10N H_2SO_4 was added and the final volume was made to 1 litre. From this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0ml solution was taken in ten different graduated test tubes. The solution in each test tube was diluted to 5ml. In each tube, 1ml molybdic acid reagent and 0.4ml of 1-amino-2-naphthol-4-sulphonic acid were added and the final volume was made up to 10ml. After 5min, the intensity of the colour was read at 620nm. A blank was run with each set of determination. A standard curve was plotted using different dilutions of potassium dihydrogen orthophosphate solution versus optical density and with the help of the standard curve, the amount of phosphorus present in the sample was determined.

3.3.6.3.2.3 Estimation of K

It was estimated with the help of flame photometer. A 10ml aliquot was taken and read by using the filter for potassium. A blank was also run side by side with each set of determination. The readings were compared with calibration curve plotted using known dilutions of standard potassium chloride solution.

3.3.6.3.2.3.1 Preparation of standard curve

1.91g potassium chloride was dissolved in 100ml DDW, and 1ml of this solution was diluted to 1 litre. This gave a solution of 10ppm potassium concentration. From this 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml solution was transferred to 10 vials separately. The solution in each vial was diluted to 10ml. The diluted solution of each vial was run separately. A blank was also run with the each set of determination. Standard curve was prepared using different dilutions of potassium chloride solution versus readings on the flame photometer.

3.3.6.3.3 Leaf protein concentration

Protein concentration was estimated by the method of Lowry *et al.* (1951). Plant material was ground to fine powder in a mortar and pestle. Five hundred mg of sample was further ground in 5ml of 5% trichloroacetate acid solution. From this, 0.1ml sample was taken in test tube and the volume was made to 1ml with DDW. Five ml of Reagent C^1 was added to the test tube and centrifuged at 4,000rpm (CPR24, New Delhi, India). Then, 0.5ml of Reagent D^2 was added to tube and mixed well. The mixture was incubated at room temperature for 30min in the dark for maximum colour development. The intensity of the blue colour developed was read at 660nm.

1. Reagent C: Prepared by mixing 50ml of Reagent A (2% sodium carbonate and 0.1N NaOH in 1:1 ratio) and 1ml of reagent B (0.5% copper sulphate and 1% potassium sodium tartrate in 1:1 ratio).
2. Reagent D: Prepared by mixing 50ml of 2% sodium carbonate solution in 1ml of reagent B.

3.3.6.3.3.1 Standard curve for protein

Fifty mg of Bovine serum albumin was dissolved in DDW in a 50ml volumetric flask and the volume was maintained. From this solution, 10ml was taken and diluted to 50ml in another 50ml volumetric flask. One ml of this solution contained 200μg protein. Different concentrations, such as 0.2, 0.4,

0.6, 0.8 and 1.0ml from this solution were taken to different test tubes and the volume was maintained to 1ml. To this, 5ml of Reagent C^1 was added, mixed well and allowed to stand for 10min followed by the addition of 0.5ml of Reagent D^2 and incubated at room temperature in the dark for 30min for maximum colour development. The colour intensity was read at 660nm.

Standard curve was plotted using different concentrations of the working standard versus optical density. With the help of this standard curve the amount of protein present in the samples was calculated.

3.3.6.4 Activities of antioxidative enzymes

The activities of antioxidative enzymes were determined in Experiment 3 and 4 at 20 and 30DAS, respectively.

Leaf samples were homogenized with an extraction buffer containing 0.5% *Triton* X-100 and 1% polyvinylpyrrolidone in 100mM potassium phosphate buffer (pH 7.0) using chilled mortar and pestle. The homogenate was centrifuged at 15000g for 20min at 4°C. The supernatant obtained after centrifugation was used for the enzymatic assays. For ascorbate peroxidase, extraction buffer was supplemented with 2mM ascorbate. The assay of following enzymes was done.

1. Catalase
2. Superoxide dismutase
3. Glutathione reductase
4. Ascorbate peroxidase

3.3.6.4.1 Catalase

The activity of catalase was measured by the method of Aebi (1984), and was determined by monitoring the disappearance of H_2O_2 at 240nm by using the extinction coefficient 0.036mM^{-1} cm^{-1}. One unit of the enzyme is the amount necessary to decompose 1μmol of H_2O_2 per minute at 25°C.

3.3.6.4.2 Superoxide dismutase

The activity of superoxide dismutase was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT), according to the methods of Beyer and Fridovich (1987) and Giannopolitis and Ries (1977). Samples were homogenized in a prechilled mortar and pestle for 2min with 1.5g of quartz sand and 10ml of homogenizing solution containing 50mM HEPES buffer and 0.1mM Na_2EDTA (pH 7.6). The homogenate was centrifuged at 15000g for 15min, and then filtered through *Whatman 42* filter paper to produce the crude extract, which was used for superoxide dismutase assay. A 5ml reaction mixture containing 50mM HEPES (pH 7.6), 0.1mM EDTA, 50mM Na_2CO_3 (pH 10.0), 13mM methionine, 0.025% *Triton* X-100, 63μmol NBT, 1.3μmol riboflavin and an enzyme extract was illuminated for 15min (360μmol m^{-2} s^{-1}) and a control set was not illuminated to correct for background absorbance. A unit of superoxide dismutase activity was defined as the amount of enzyme required to cause 50% inhibition of the reaction of NBT at 560nm.

3.3.6.4.3 Glutathione reductase

The activity of glutathione reductase was determined by the method described by Foyer and Halliwell (1976) by monitoring the glutathione dependent oxidation of NADPH at 340nm. The assay mixture contained 25mM phosphate buffer (pH 7.8), 0.5mM oxidized glutathione (GSSG), 0.2mM NADPH and the enzyme extract. The activity of GR was calculated by using extinction coefficient 6.2mM^{-1} cm^{-1}. One unit of enzyme is the amount necessary to decompose 1μmol of NADPH per minute at 25°C.

3.3.6.4.4 Ascorbate peroxidase

The activity of ascorbate peroxidase was determined according to Nakano and Asada (1981) by the decrease in absorbance of ascorbate at 290nm. The assay mixture contained 50mM phosphate buffer (pH 7.0), 0.1mM EDTA, 0.5mM ascorbate, 0.1mM H_2O_2 and enzyme extract. Ascorbate

peroxidase activity was calculated by using the extinction coefficient 2.8 mM^{-1} cm^{-1}. One unit of the enzyme is the amount necessary to decompose 1μmol of substrate per minute at 25°C.

3.3.6.5 Yield characteristics

Yield is the final manifestation of morphological, physiological and biochemical traits of a crop, which are dependent upon various environmental factors.

At harvest following parameters were recorded.

1. Pod length
2. Pod number per plant
3. Seed number per pod
4. Seed yield per plant

At harvest, pods were collected and counted. Pod length was measured on a meter scale. The number of seeds from each pod was counted. The total seeds from a plant in each treatment were cleared, sun-dried and weighed to compute seed yield per plant.

3.3.7 Data Analysis

Data were statistically analyzed using analysis of variance (ANOVA) by SPSS ver. 10 Inc., Chicago, USA. The least significant difference (LSD) was calculated for the significant data at $P<0.05$. The treatment means were separated by Duncan's Multiple Range Test (DMRT).

EXPERIMENTAL RESULTS

Contents

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The chapter 'Experimental Results' reports results on the observations recorded for different growth, photosynthetic and biochemical characteristics, activities of antioxidative enzymes and yield characteristics. The details of the determinations have been described in the chapter 'Materials and Methods'. As mentioned earlier, Experiments 1 and 3 were conducted on mungbean, a short duration crop. Therefore, the observations were recorded at 20, 40 and 60DAS. Experiments 2 and 4 were conducted on mustard, and the observations were recorded at 30, 60, 90 and 120DAS.

4.1 Experiment 1

The aim of the experiment was to assess the influence of 0, 50 and 100mM NaCl salinity stress on growth, photosynthetic and yield characteristics of four cultivars, Pusa Vishal, PDM54, T44 and Tram, of mungbean, and select the salinity tolerant and salinity non-tolerant cultivars on the basis of their performance. Growth and photosynthetic characteristics were observed at 20 and 40DAS and yield characteristics at 60DAS.

4.1.1 Growth characteristics

Growth of all the cultivars decreased with the increasing salinity levels at all the sampling times (Tables 4-10). At initial stage of growth, i.e. 20DAS, the cultivars did not respond significantly to salinity treatment, except for plant dry mass. However, at 40DAS, the effect of salinity on the growth of the cultivars was significant. Maximum decrease in growth resulted with the application of 100mM NaCl.

Among cultivars, Tram exhibited greatest decrease in the growth characteristics due to salinity stress, and Pusa Vishal showed lowest decrease followed by PDM54 and T44. The per cent decrease in the growth characteristics of Pusa Vishal and PDM54 were less compared to T44 and Tram. In Pusa Vishal, the treatment of 50mM NaCl and 100mM NaCl

Table 4: Root length (cm plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	16.50	11.30	6.20	11.33^{c}
	Pusa Vishal	22.80	18.60	14.40	18.60^{a}
	Tram	14.10	9.30	4.60	9.33^{d}
	PDM54	20.20	16.30	12.50	16.33^{b}
	Mean	18.40	13.88*	9.43*	
	LSD at $P<0.05$	S = 1.16	C = 1.34	S×C = NS	
40	T44	29.60*c*	21.70*e*	14.10*f*	21.80^{c}
	Pusa Vishal	39.10*a*	33.90*b*	28.60*c*	33.87^{a}
	Tram	28.30*cd*	20.30*e*	11.80*g*	20.13^{d}
	PDM54	38.00*a*	32.50*b*	27.20*d*	32.57^{b}
	Mean	33.75	27.10*	20.43*	
	LSD at $P<0.05$	S = 0.80	C = 0.93	S×C = 1.60	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 5: Root fresh mass (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	0.84	0.58	0.31	0.58^{c}
	Pusa Vishal	1.15	0.94	0.73	0.94^{a}
	Tram	0.76	0.50	0.25	0.50^{d}
	PDM54	1.04	0.84	0.64	0.84^{b}
	Mean	0.95	0.71*	0.48*	
	LSD at $P<0.05$	S = 0.07	C = 0.08	S×C = NS	
40	T44	2.42*bc*	1.78*f*	1.13*h*	1.77^{c}
	Pusa Vishal	2.92*a*	2.53*b*	2.13*d*	2.53^{a}
	Tram	2.32*c*	1.64*g*	0.96*i*	1.64^{d}
	PDM54	2.82*a*	2.42*b*	2.01*e*	2.42^{b}
	Mean	2.62	2.09*	1.56*	
	LSD at $P<0.05$	S = 0.06	C = 0.07	S×C = 0.11	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$

Table 6: Root dry mass (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	0.19	0.13	0.07	0.13^{c}
	Pusa Vishal	0.29	0.24	0.19	0.24^{a}
	Tram	0.16	0.10	0.05	0.10^{d}
	PDM54	0.26	0.21	0.16	0.21^{b}
	Mean	0.22	0.17*	0.12*	
	LSD at P<0.05	S = 0.01	C = 0.01	S×C = NS	
40	T44	0.57*d*	0.42*f*	0.28*h*	0.42^{c}
	Pusa Vishal	0.80*a*	0.70*b*	0.59*d*	0.70^{a}
	Tram	0.51*e*	0.36*g*	0.21*i*	0.36^{d}
	PDM54	0.74*b*	0.63*c*	0.53*e*	0.63^{b}
	Mean	0.65	0.53*	0.40*	
	LSD at P<0.05	S = 0.02	C = 0.02	S×C = 0.03	

Different letters indicate significant difference in the interaction values of salinity and cultivar at P<0.05.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at P<0.05.

Table 7: Leaf fresh mass (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	0.80	0.55	0.30	0.55^{c}
	Pusa Vishal	1.20	0.98	0.77	0.98^{a}
	Tram	0.71	0.47	0.23	0.47^{d}
	PDM54	1.10	0.88	0.67	0.88^{b}
	Mean	0.95	0.72*	0.49*	
	LSD at $P<0.05$	S = 0.06	C = 0.07	S×C = NS	
40	T44	6.87*b*	5.04*de*	3.27*f*	5.06^{b}
	Pusa Vishal	7.72*a*	6.68*b*	5.69*c*	6.70^{a}
	Tram	6.62*b*	4.69*e*	2.73*f*	4.68^{c}
	PDM54	7.55*a*	6.45*b*	5.40*cd*	6.46^{a}
	Mean	7.19	5.72*	4.27*	
	LSD at $P<0.05$	S = 0.30	C = 0.34	S×C = 0.59	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 8: Leaf dry mass (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	0.21	0.14	0.08	0.15^{c}
	Pusa Vishal	0.35	0.29	0.22	0.29^{a}
	Tram	0.18	0.12	0.06	0.12^{d}
	PDM54	0.32	0.26	0.19	0.25^{b}
	Mean	0.26	0.20*	0.14*	
	LSD at P<0.05	S = 0.01	C = 0.02	S×C = NS	
40	T44	1.64*cd*	1.21*g*	0.79*i*	1.22^{c}
	Pusa Vishal	2.03*a*	1.76*b*	1.50*e*	1.76^{a}
	Tram	1.56*de*	1.12*h*	0.65*j*	1.11^{d}
	PDM54	1.95*a*	1.67*c*	1.40*f*	1.67^{b}
	Mean	1.79	1.44*	1.09*	
	LSD at P<0.05	S = 0.04	C = 0.05	S×C = 0.08	

Different letters indicate significant difference in the interaction values of salinity and cultivar at P<0 05
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at P<0 05

Table 9: Leaf area (cm^2 $plant^{-1}$) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	42.60	29.40	15.40	29.13^{b}
	Pusa Vishal	50.50	41.20	32.10	41.27^{a}
	Tram	39.70	26.20	12.40	26.10^{c}
	PDM54	48.70	39.40	29.90	39.33^{a}
	Mean	45.38	34.05*	22.45*	
	LSD at $P<0.05$	S = 2.90	C = 3.35	S×C = NS	
40	T44	143.90*c*	105.30*g*	70.30*i*	106.50^{c}
	Pusa Vishal	166.50*a*	144.40*c*	122.90*e*	144.60^{a}
	Tram	138.50*cd*	98.10*h*	60.20*j*	98.93^{d}
	PDM54	160.20*b*	137.40*d*	114.80*f*	137.47^{b}
	Mean	152.28	121.30*	92.05*	
	LSD at $P<0.05$	S = 3.01	C = 3.48	S×C = 6.03	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 10: Plant dry mass (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	1.00*b*	0.69*ef*	0.36*h*	0.68^{c}
	Pusa Vishal	1.14*a*	0.93*cd*	0.72*e*	0.93^{a}
	Tram	0.96*bc*	0.63*g*	0.30*i*	0.63^{d}
	PDM54	1.09*a*	0.88*d*	0.67*fg*	0.88^{b}
	Mean	1.05	0.78*	0.51*	
	LSD at $P<0.05$	S = 0.02	C = 0.03	S×C = 0.05	
40	T44	3.19*b*	2.33*e*	1.51*g*	2.34^{c}
	Pusa Vishal	3.61*a*	3.13*b*	2.63*d*	3.12^{a}
	Tram	3.10*bc*	2.19*f*	1.30*h*	2.20^{d}
	PDM54	3.53*a*	3.02*c*	2.52*d*	3.02^{b}
	Mean	3.36	2.67*	1.99*	
	LSD at $P<0.05$	S = 0.07	C = 0.08	S×C = 0.13	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

decreased root length by 18.42 and 36.84%, root fresh mass by 18.26 and 36.52%, root dry mass by 17.24 and 34.48%, leaf fresh mass by 18.33 and 35.83%, leaf dry mass by 17.14 and 37.14%, leaf area by 18.42 and 36.44% and plant dry mass by 18.42 and 36.84% at 20DAS in comparison to control. At 40DAS, the decreases in the above characteristics due to 50mM NaCl and 100mM NaCl were 13.30 and 26.85%, 13.36 and 27.05%, 12.50 and 26.25%, 13.47 and 26.30%, 13.30 and 26.11%, 13.27 and 26.19% and 13.30 and 27.15%, respectively in comparison to control.

The decreases in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass in Tram with 50mM NaCl and 100mM NaCl were 34.04 and 67.38%, 34.21 and 67.11%, 37.50 and 68.75%, 33.80 and 67.61%, 33.33 and 66.67%, 34.01 and 68.77% and 34.38 and 68.75% at 20DAS, and 28.27 and 58.30%, 29.31 and 58.62%, 29.41 and 58.82%, 29.15 and 58.76%, 28.21 and 58.33%, 29.17 and 56.53% and 29.35 and 58.06%, respectively at 40DAS. The order of the growth performance of the cultivars was: Pusa Vishal > PDM54 > T44 > Tram.

4.1.2 Photosynthetic characteristics

Among photosynthetic characteristics observed, carbonic anhydrase activity and net photosynthetic rate decreased with the increasing salinity levels, and the effect of 100mM NaCl was more conspicuous on all the cultivars at both the sampling times (Tables 11-12).

Carbonic anhydrase activity and net photosynthetic rate decreased maximally in Tram followed by T44 at 20 and 40DAS. The other two cultivars responded equally to NaCl treatment in respect of photosynthetic characteristics. In Pusa Vishal, carbonic anhydrase activity and net photosynthetic rate decreased by 36.05 and 12.35% due to 50mM NaCl and 40.03 and 34.25% due to 100mM NaCl at 20DAS, and 11.23 and 11.76% due to 50mM NaCl and 13.75 and 28.41% due to 100mM NaCl at 40DAS.

Table 11: Carbonic anhydrase activity (m mol m^{-2} leaf s^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	10.23*c*	6.30*g*	4.07*j*	6.87^{c}
	Pusa Vishal	11.79*a*	7.54*e*	7.07*f*	8.80^{a}
	Tram	9.75*d*	5.54*h*	2.86*k*	6.05^{d}
	PDM54	11.25*b*	7.11*f*	5.03*i*	7.80^{b}
	Mean	10.76	6.62*	4.76*	
	LSD at $P<0.05$	S = 0.06	C = 0.07	S×C = 0.13	
40	T44	14.24*c*	11.54*h*	9.34*j*	11.71^{c}
	Pusa Vishal	15.85*a*	14.07*cd*	13.67*e*	14.53^{a}
	Tram	13.88*de*	11.07*i*	8.66*k*	11.20^{d}
	PDM54	15.28*b*	12.83*f*	12.02*g*	13.38^{b}
	Mean	14.81	12.38*	10.92*	
	LSD at $P<0.05$	S = 0.13	C = 0.14	S×C = 0.25	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 12: Net photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
20	T44	15.36*e*	12.50*i*	9.33*k*	12.40[c]
	Pusa Vishal	21.78*a*	19.09*c*	14.32*g*	18.40[a]
	Tram	14.78*f*	11.72*j*	8.92*k*	11.81[d]
	PDM54	20.54*b*	16.77*d*	13.34*h*	16.88[b]
	Mean	18.12	15.02*	11.48*	
	LSD at $P<0.05$	S = 0.22	C = 0.26	S×C = 0.44	
40	T44	18.52*e*	15.45*g*	12.31*i*	15.43[c]
	Pusa Vishal	23.90*a*	21.09*c*	17.11*f*	20.70[a]
	Tram	17.34*f*	14.15*h*	11.40*j*	14.30[d]
	PDM54	22.96*b*	19.94*d*	15.59*g*	19.50[b]
	Mean	20.68	17.66*	14.10*	
	LSD at $P<0.05$	S = 0.22	C = 0.25	S×C = 0.44	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

The decreases in carbonic anhydrase activity and net photosynthetic rate in Tram with 50mM NaCl and 100mM NaCl were 43.18 and 20.70% and 70.67 and 39.65% at 20DAS, and 20.24 and 18.40% and 37.61 and 34.26% at 40DAS, respectively. Photosynthetic characteristics in the cultivars were in the order: Pusa Vishal > PDM54 > T44 > Tram.

4.1.3 Yield characteristics

Among yield characteristics, pod length, pod number per plant, seed number per pod and seed yield were noted. Plants treated with 50mM NaCl exhibited a significant decrease over control on the yield characteristics (Table 13). At 100mM NaCl treatment, the plants did not survive up to maturity stage and the yield characteristics, therefore, could not be recorded. The cultivars responded differently to NaCl treatment for yield characteristics compared to growth and photosynthetic characteristics. The cultivars T44 and Tram exhibited about equal and greatest decrease in yield characteristics, whereas Pusa Vishal and PDM54 showed lowest decrease. The decrease in pod length, pod number, seed number and seed yield of Pusa Vishal and Tram was 13.37, 13.26, 13.33 and 13.45% and 29.47, 29.15, 28.70 and 28.63%, respectively due to 50mM NaCl in comparison to control. The order of performance of cultivars for the yield characteristics was: Pusa Vishal > PDM54 > T44 > Tram.

4.2 Experiment 2

In this experiment the influence of 0, 50 and 100mM NaCl was studied on growth, photosynthetic and yield characteristics of Alankar, Pusa Bold, Sakha and PBM16 cultivars of mustard. On the basis of their performance under salinity treatment, the cultivars were categorized as salinity tolerant and salinity non-tolerant. The observations on growth and photosynthesis were recorded at 30, 60 and 90DAS and yield at 120DAS.

4.2.1 Growth characteristics

The effect of salinity on growth was found significant at all sampling times. The effect of salinity and cultivar interaction was also found significant

Table 13: Pod length (cm), pod number plant^{-1}, seed number pod^{-1} and seed yield (g plant^{-1}) of four cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at harvest, i.e., 60 days after sowing (DAS).

		Salinity (S) level (mM NaCl)		
DAS	Cultivar (C)	0	50	Mean
			Pod length	
60	T44	6.27*c*	4.63*g*	5.45^{c}
	Pusa Vishal	6.73*a*	5.83*e*	6.28^{a}
	Tram	6.04*d*	4.26*h*	5.15^{d}
	PDM54	6.54*b*	5.61*f*	6.08^{b}
	Mean	6.40	5.08*	
	LSD at $P<0.05$	S = 0.07	C = 0.10	S×C = 0.15
			Pod number	
60	T44	26.40*e*	19.40*g*	22.90^{c}
	Pusa Vishal	36.20*a*	31.40*c*	33.80^{a}
	Tram	24.70*f*	17.50*h*	21.10^{d}
	PDM54	34.50*b*	29.60*d*	32.05^{b}
	Mean	30.45	24.48*	
	LSD at $P<0.05$	S = 0.55	C = 0.78	S×C = 1.11
			Seed number	
60	T44	11.10*c*	8.20*g*	9.65^{c}
	Pusa Vishal	12.00*a*	10.40*e*	11.20^{a}
	Tram	10.80*d*	7.70*h*	9.25^{d}
	PDM54	11.80*b*	10.10*f*	10.95^{b}
	Mean	11.43	9.10*	
	LSD at $P<0.05$	S = 0.08	C = 0.11	S×C = 0.16
			Seed yield	
60	T44	7.66*d*	5.67*f*	6.67^{c}
	Pusa Vishal	8.92*a*	7.72*c*	8.32^{a}
	Tram	7.16*e*	5.11*g*	6.14^{d}
	PDM54	8.65*b*	7.57*d*	8.11^{b}
	Mean	8.10	6.52*	
	LSD at $P<0.05$	S = 0.12	C = 0.17	S×C = 0.24

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

except for leaf fresh and dry mass and plant dry mass at initial stage of growth (Tables 14-20).

The increase in salinity levels decreased the growth characteristics of all the cultivars. The observations recorded at all the sampling times showed similar pattern of cultivar response to NaCl concentrations.

Growth reductions in Sakha and PBM16 were significantly greater than in Alankar and Pusa Bold with NaCl concentrations. Maximum reductions in growth were noted with 100mM NaCl.

In Alankar, the treatment of 100mM NaCl reduced root length by 47.84, 36.79 and 27.45%, root fresh mass by 47.47, 37.91 and 26.42%, root dry mass by 47.83, 38.07 and 26.76%, leaf fresh mass by 47.03, 37.03 and 27.84%, leaf dry mass by 46.98, 37.44 and 26.60%, leaf area by 47.16, 36.75 and 27.99% and plant dry mass by 47.57, 37.78 and 26.42% at 30, 60 and 90DAS, respectively over control.

Contrarily, higher decrease in growth was shown by PBM16. Regarding per cent decreases in PBM16 due to 100mM NaCl, root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass were reduced by 78.28, 79.06, 78.26, 76.65, 76.22, 76.86 and 78.44%, respectively at 30DAS. The decreases in these characteristics at 60DAS were 68.92, 67.58, 68.26, 68.62, 66.82, 66.67 and 67.60%, and were 56.43, 56.94, 57.37, 57.25, 58.03, 56.05 and 56.42%, respectively at 90DAS.

4.2.2 Photosynthetic characteristics

Photosynthetic characteristics declined significantly with the increasing salinity levels at all the sampling times (Tables 21-22). The data indicate progressive decrease with the increasing salinity in all the cultivars. The carbonic anhydrase activity and net photosynthetic rate in salinity treatments

Table 14: Root length (cm plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	25.50*a*	19.50*c*	13.30*e*	19.43[a]
	Pusa Bold	25.10*a*	19.00*c*	12.80*e*	18.97[a]
	Sakha	22.60*b*	14.60*d*	6.20*f*	14.47[b]
	PBM16	22.10*b*	13.40*e*	4.80*g*	13.43[c]
	Mean	23.83	16.63*	9.28*	
	LSD at $P<0.05$	S = 0.47	C = 0.55	S×C = 0.95	
60	Alankar	31.80*a*	25.90*b*	20.10*c*	25.93[a]
	Pusa Bold	31.40*a*	25.30*b*	19.40*c*	25.37[a]
	Sakha	26.10*b*	18.00*d*	9.40*f*	17.83[b]
	PBM16	25.10*b*	16.70*e*	7.80*g*	16.53[c]
	Mean	28.60	21.48*	14.18*	
	LSD at $P<0.05$	S = 0.53	C = 0.61	S×C = 1.06	
90	Alankar	41.90*a*	36.30*c*	30.40*d*	36.20[a]
	Pusa Bold	41.80*a*	35.80*c*	29.70*de*	35.77[a]
	Sakha	39.00*b*	28.60*e*	18.20*g*	28.60[b]
	PBM16	38.10*b*	27.30*f*	16.60*h*	27.33[c]
	Mean	40.20	32.00*	23.73*	
	LSD at $P<0.05$	S = 0.55	C = 0.64	S×C = 1.11	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 15: Root fresh mass (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	6.13*a*	4.68*c*	3.22*e*	4.68[a]
	Pusa Bold	5.49*b*	4.15*d*	2.82*f*	4.15[b]
	Sakha	2.82*f*	1.79*h*	0.79*j*	1.80[c]
	PBM16	2.34*g*	1.45*i*	0.49*k*	1.43[d]
	Mean	4.19	3.02*	1.83*	
	LSD at $P<0.05$	S = 0.14	C = 0.16	S×C = 0.27	
60	Alankar	11.00*a*	8.99*c*	6.83*f*	8.94[a]
	Pusa Bold	10.03*b*	8.12*d*	6.19*g*	8.11[b]
	Sakha	8.07*d*	5.49*h*	3.08*j*	5.55[c]
	PBM16	7.28*e*	4.78*i*	2.36*k*	4.81[d]
	Mean	*9.10*	*6.85**	*4.61**	
	LSD at $P<0.05$	S = 0.19	C = 0.22	S×C = 0.38	
90	Alankar	15.48*a*	13.41*c*	11.39*e*	13.42[a]
	Pusa Bold	14.48*b*	12.38*d*	10.16*f*	12.34[b]
	Sakha	11.13*e*	8.27*g*	5.39*i*	8.26[c]
	PBM16	9.66*f*	6.90*h*	4.16*j*	6.91[d]
	Mean	12.69	10.24*	7.78*	
	LSD at $P<0.05$	S = 0.27	C = 0.31	S×C = 0.53	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 16: Root dry mass (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	1.84*a*	1.41*c*	0.96*e*	1.40[a]
	Pusa Bold	1.62*b*	1.23*d*	0.82*f*	1.22[b]
	Sakha	0.85*f*	0.53*h*	0.22*j*	0.53[c]
	PBM16	0.69*g*	0.43*i*	0.15*k*	0.42[d]
	Mean	1.25	0.90*	0.54*	
	LSD at $P<0.05$	S = 0.04	C = 0.05	S×C = 0.08	
60	Alankar	3.52*a*	2.87*c*	2.18*f*	2.86[a]
	Pusa Bold	3.20*b*	2.59*d*	1.95*g*	2.58[b]
	Sakha	2.55*d*	1.72*h*	0.98*j*	1.75[c]
	PBM16	2.30*e*	1.51*i*	0.73*k*	1.51[d]
	Mean	2.89	2.17*	1.46*	
	LSD at $P<0.05$	S = 0.06	C = 0.07	S×C = 0.12	
90	Alankar	5.12*a*	4.43*c*	3.75*e*	4.43[a]
	Pusa Bold	4.78*b*	4.09*d*	3.35*f*	4.07[b]
	Sakha	3.67*e*	2.72*g*	1.74*i*	2.71[c]
	PBM16	3.19*f*	2.25*h*	1.36*j*	2.27[d]
	Mean	4.19	3.37*	2.55*	
	LSD at $P<0.05$	S = 0.09	C = 0.10	S×C = 0.18	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 17: Leaf fresh mass (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	10.93	8.36	5.78	8.36[a]
	Pusa Bold	10.21	7.73	5.28	7.74[b]
	Sakha	7.31	4.58	2.00	4.63[c]
	PBM16	6.38	3.86	1.49	3.91[d]
	Mean	8.71	6.13*	3.64*	
	LSD at $P<0.05$	S = 0.21	C = 0.25	S×C = NS	
60	Alankar	20.58*a*	16.83*d*	12.96*f*	16.79[a]
	Pusa Bold	19.82*b*	15.99*e*	12.10*g*	15.97[b]
	Sakha	17.62*c*	12.02*g*	6.85*i*	12.16[c]
	PBM16	16.92*d*	11.22*h*	5.31*j*	11.15[d]
	Mean	18.74	14.02*	9.31*	
	LSD at $P<0.05$	S = 0.32	C = 0.37	S×C = 0.65	
90	Alankar	26.11*a*	22.62*b*	18.84*d*	22.52[a]
	Pusa Bold	25.48*a*	21.83*c*	17.89*e*	21.73[b]
	Sakha	23.04*b*	16.89*f*	10.89*h*	16.94[c]
	PBM16	22.41*bc*	16.00*g*	9.58*i*	16.00[d]
	Mean	24.26	19.34*	14.30*	
	LSD at $P<0.05$	S = 0.36	C = 0.41	S×C = 0.72	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 18: Leaf dry mass (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	2.81	2.15	1.49	2.15[a]
	Pusa Bold	2.63	1.98	1.36	1.99[b]
	Sakha	1.88	1.18	0.52	1.20[c]
	PBM16	1.64	1.00	0.39	1.01[d]
	Mean	2.24	1.58*	0.94*	
	LSD at $P<0.05$	S = 0.05	C = 0.06	S×C = NS	
60	Alankar	5.77*a*	4.71*c*	3.61*e*	4.70[a]
	Pusa Bold	5.45*b*	4.40*d*	3.29*f*	4.38[b]
	Sakha	4.76*c*	3.27*f*	1.83*h*	3.29[c]
	PBM16	4.46*d*	2.93*g*	1.48*i*	2.96[d]
	Mean	5.11	3.83*	2.55*	
	LSD at $P<0.05$	S = 0.09	C = 0.11	S×C = 0.18	
90	Alankar	8.12*a*	7.04*c*	5.96*f*	7.04[a]
	Pusa Bold	7.74*b*	6.64*d*	5.43*g*	6.60[b]
	Sakha	6.64*d*	4.82*h*	3.15*j*	4.87[c]
	PBM16	6.29*e*	4.43*i*	2.64*k*	4.45[d]
	Mean	7.20	5.73*	4.30*	
	LSD at $P<0.05$	S = 0.12	C = 0.14	S×C = 0.24	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 19: Leaf area (cm^2 $plant^{-1}$) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	564.00*a*	432.00*e*	298.00*h*	431.33[a]
	Pusa Bold	538.00*b*	408.00*f*	271.00*i*	405.67[b]
	Sakha	497.00*c*	318.00*g*	138.00*j*	317.67[c]
	PBM16	471.00*d*	289.00*hi*	109.00*k*	289.67[d]
	Mean	517.50	361.75*	204.00*	
	LSD at $P<0.05$	S = 9.96	C = 11.50	S×C = 19.92	
60	Alankar	762.00*a*	623.00*d*	482.00*e*	622.33[a]
	Pusa Bold	744.00*a*	600.00*d*	453.00*fg*	599.00[b]
	Sakha	698.00*b*	473.00*ef*	263.00*h*	478.00[c]
	PBM16	672.00*c*	443.00*g*	224.00*i*	446.33[d]
	Mean	719.00	534.75*	355.50*	
	LSD at $P<0.05$	S = 11.68	C = 13.48	S×C = 23.35	
90	Alankar	1086.00*a*	943.00*b*	782.00*d*	937.00[a]
	Pusa Bold	1063.00*a*	908.00*c*	746.00*e*	905.67[b]
	Sakha	903.00*c*	664.00*f*	432.00*h*	666.33[c]
	PBM16	885.00*c*	632.00*g*	389.00*i*	635.33[d]
	Mean	984.25	786.75*	587.25*	
	LSD at $P<0.05$	S = 15.56	C = 17.97	S×C = 31.12	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 20: Plant dry mass (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	6.39	4.89	3.35	4.88[a]
	Pusa Bold	5.87	4.43	2.97	4.42[b]
	Sakha	4.37	2.71	1.14	2.74[c]
	PBM16	3.85	2.39	0.83	2.36[d]
	Mean	5.12	3.61*	2.07*	
	LSD at $P<0.05$	S = 0.12	C = 0.14	S×C = NS	
60	Alankar	14.40*a*	11.74*d*	8.96*f*	11.70[a]
	Pusa Bold	13.92*b*	11.20*e*	8.50*fg*	11.20[b]
	Sakha	12.30*c*	8.38*g*	4.72*i*	8.47[c]
	PBM16	11.82*d*	7.83*h*	3.83*j*	7.83[d]
	Mean	13.11	9.78*	6.50*	
	LSD at $P<0.05$	S = 0.23	C = 0.26	S×C = 0.45	
90	Alankar	21.99*a*	19.03*c*	16.18*e*	19.06[a]
	Pusa Bold	21.53*a*	18.40*d*	15.24*f*	18.39[b]
	Sakha	20.09*b*	15.02*f*	9.26*h*	14.79[c]
	PBM16	19.62*b*	13.98*g*	8.55*i*	14.05[d]
	Mean	20.81	16.61*	12.31*	
	LSD at $P<0.05$	S = 0.29	C = 0.34	S×C = 0.58	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

were lower as compared to control. The greatest decrease was observed at the highest level of NaCl concentration.

Alankar had significantly greater carbonic anhydrase activity and net photosynthetic rate than PBM16 followed by Sakha at 0, 50 and 100mM NaCl concentrations. In Alankar, the decrease in carbonic anhydrase activity and net photosynthetic rate with 50mM and 100mM NaCl was 18.12 and 13.15% and 29.00 and 35.93% at 30DAS; 11.24 and 11.94% and 13.72 and 28.82% at 60DAS, and 10.05 and 9.32% and 12.06 and 26.25% respectively at 90DAS.

In PBM16, the carbonic anhydrase activity and net photosynthetic rate decreased by 26.07 and 22.93% due to 50mM NaCl and 48.80 and 42.35% with 100mM NaCl at 30DAS; 20.24 and 18.60% and 37.66 and 34.94% with 50mM and 100mM NaCl at 60DAS, and 19.05 and 16.32% and 36.04 and 33.19% with 50mM and 100mM NaCl, respectively at 90DAS.

4.2.3 Yield characteristics

Yield characteristics were affected by salinity stress in all the cultivars. In pod length, the interaction effect of cultivar and salinity was found non-significant (Table 23).. Yield and its attributing characteristics decreased with 50mM NaCl in all the cultivars. The treatment of 100mM NaCl proved deleterious and plants did not survive up to maturity under this treatment. Sakha and PBM16 were very sensitive to salinity stress, therefore, produced lesser yield than Alankar and Pusa Bold.

Treatment of 50mM NaCl caused 11.76, 13.21, 13.31 and 13.21% reduction in pod length, pod number, seed number and seed yield in Alankar. The above characteristics decreased by 30.23, 29.58, 28.17 and 28.45% in PBM16, respectively.

Table 21: Carbonic anhydrase activity (m mol m^{-2} leaf s^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	13.69*a*	11.21*e*	9.72*g*	11.54[a]
	Pusa Bold	13.24*b*	10.29*f*	8.36*j*	10.63[b]
	Sakha	12.18*c*	9.19*h*	7.39*k*	9.59[c]
	PBM16	11.70*d*	8.65*i*	5.99*l*	8.78[d]
	Mean	12.70	9.84*	7.87*	
	LSD at $P<0.05$	S = 0.08	C = 0.09	S×C = 0.16	
60	Alankar	17.79*a*	15.79*d*	15.35*e*	16.31[a]
	Pusa Bold	17.46*b*	14.66*f*	13.74*g*	15.29[b]
	Sakha	16.35*c*	13.25*h*	10.72*j*	13.44[c]
	PBM16	16.01*d*	12.77*i*	9.98*k*	12.92[d]
	Mean	16.90	14.12*	12.45*	
	LSD at $P<0.05$	S = 0.12	C = 0.14	S×C = 0.25	
90	Alankar	16.92*a*	15.22*c*	14.88*d*	15.67[a]
	Pusa Bold	16.69*a*	14.18*e*	13.34*f*	14.74[b]
	Sakha	15.52*b*	12.88*g*	10.39*i*	12.93[c]
	PBM16	15.01*cd*	12.15*h*	9.60*j*	12.25[d]
	Mean	16.04	13.61*	12.05*	
	LSD at $P<0.05$	S = 0.12	C = 0.14	S×C = 0.24	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 22: Net photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

		Salinity (S) level (mM NaCl)			
DAS	Cultivar (C)	0	50	100	Mean
30	Alankar	22.74*a*	19.75*c*	14.57*f*	19.02[a]
	Pusa Bold	21.29*b*	17.74*de*	13.33*g*	17.45[b]
	Sakha	18.23*d*	14.47*f*	10.88*h*	14.53[c]
	PBM16	17.31*e*	13.34*g*	9.98*i*	13.54[d]
	Mean	19.89	16.33*	12.19*	
	LSD at $P<0.05$	S = 0.25	C = 0.29	S×C = 0.50	
60	Alankar	25.05*a*	22.06*c*	17.83*f*	21.65[a]
	Pusa Bold	23.72*b*	20.58*d*	16.12*h*	20.14[b]
	Sakha	20.38*d*	17.05*g*	13.52*i*	16.98[c]
	PBM16	19.46*e*	15.84*h*	12.66*j*	15.99[d]
	Mean	22.15	18.88*	15.03*	
	LSD at $P<0.05$	S = 0.20	C = 0.23	S×C = 0.40	
90	Alankar	22.97*a*	20.83*c*	16.94*f*	20.25[a]
	Pusa Bold	21.61*b*	19.08*d*	15.16*h*	18.62[b]
	Sakha	18.90*d*	16.33*g*	12.81*i*	16.01[c]
	PBM16	18.02*e*	15.08*h*	12.04*j*	15.05[d]
	Mean	20.38	17.83*	14.24*	
	LSD at $P<0.05$	S = 0.19	C = 0.22	S×C = 0.38	

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

Table 23: Pod length (cm), pod number plant^{-1}, seed number pod^{-1} and seed yield (g plant^{-1}) of four cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at harvest, i.e., 120 days after sowing (DAS).

		Salinity (S) level (mM NaCl)		
DAS	Cultivar (C)	0	50	Mean
			Pod length	
120	Alankar	5.10	4.50	4.80[a]
	Pusa Bold	4.90	4.20	4.55[b]
	Sakha	4.50	3.30	3.90[c]
	PBM16	4.30	3.00	3.65[d]
	Mean	4.70	3.75*	
	LSD at $P<0.05$	S = 0.22	C = 0.31	S×C = NS
			Pod number	
120	Alankar	108.56*a*	94.22*cd*	101.39[a]
	Pusa Bold	103.49*b*	88.69*e*	96.09[b]
	Sakha	95.39*c*	69.56*f*	82.48[c]
	PBM16	92.30*d*	65.00*g*	78.65[d]
	Mean	99.94	79.37*	
	LSD at $P<0.05$	S = 0.99	C = 1.40	S×C = 1.98
			Seed number	
120	Alankar	12.55*a*	10.88*d*	11.72[a]
	Pusa Bold	12.24*b*	10.48*e*	11.36[b]
	Sakha	11.25*c*	8.24*f*	9.75[c]
	PBM16	10.90*d*	7.83*g*	9.37[d]
	Mean	11.74	9.36*	
	LSD at $P<0.05$	S = 0.13	C = 0.09	S×C = 19.00
			Seed yield	
120	Alankar	6.36*a*	5.52*c*	5.94[a]
	Pusa Bold	5.92*b*	5.07*d*	5.50[b]
	Sakha	5.01*d*	3.68*f*	4.35[c]
	PBM16	4.71*e*	3.37*g*	4.04[d]
	Mean	5.50	4.41*	
	LSD at $P<0.05$	S = 0.09	C = 0.13	S×C = 0.19

Different letters indicate significant difference in the interaction values of salinity and cultivar at $P<0.05$.
* indicates significant effect of salinity treatment in comparison to control.
The superscript letters on the values in the column of cultivar Mean show significant difference at $P<0.05$.

4.3 Experiments 3 and 4

The experiments were conducted based on the findings of Experiments 1 and 2. The aim of the experiments was to study the effect of salicylic acid (SA) in alleviating the salinity stress effects in mungbean (Experiment 3) and mustard (Experiment 4) and the SA-mediated mechanisms responsible for tolerance against salinity stress. As described earlier Pusa Vishal and Tram cultivars of mungbean emerged as salinity-tolerant and salinity non-tolerant, and Alankar as tolerant and PBM16 as non-tolerant cultivars of mustard. It has also been detailed out that the treatment of 100mM NaCl was found deleterious for yield of both the crop plants as the treatment caused major injury. Therefore, the plants could not survive up to maturity stage. Therefore, in Experiment 3 and Experiment 4, 100mM NaCl treatment was not included in the study. These experiments were designed to study the effect of 0.0, 0.1, 0.5 and 1.0mM SA applied exogenously as foliar spray on tolerant and non-tolerant cultivars grown with 0 or 50mM NaCl. In these two experiments growth, photosynthetic, biochemical and yield characteristics were studied. The timing of samplings for these characteristics was 20, 40 and 60DAS for mungbean, and 30, 60, 90 and 120DAS for mustard. The activities of antioxidaive enzymes were also measured in these two experiments at 20DAS in mungbean and 30DAS in mustard. The results noted for both the experiments have been described in detail in the following pages.

4.3.1 Experiment 3

4.3.1.1 Growth characteristics

The effects of SA application on growth were found significant, except for root fresh mass for non-tolerant (Tram) cultivar at 40DAS. SA application increased growth characteristics of both the cultivars grown under non-saline (control) condition, and ameliorating salinity stress effects was also observed.

For both the cultivars, the application of 0.5mM SA proved most effective in enhancing growth under normal and salinity stress conditions.

The application of 0.5mM SA on Pusa Vishal and Tram under non-saline condition increased root length by 18.92 and 14.49% at 20DAS, and 19.90 and 15.71% at 40DAS; root fresh mass by 19.17 and 14.81% at 20DAS, and 20.13 and 15.97% at 40DAS; root dry mass by 19.35 and 18.75% at 20DAS, and 20.73 and 15.09% at 40DAS; leaf fresh mass by 19.35 an 14.29% at 20DAS, and 20.00 and 15.95% at 40DAS; leaf dry mass by 18.92 and 15.00% at 20DAS, and 20.00 and 15.82% at 40DAS; leaf area by 18.96 and 14.97% at 20DAS, and 19.96 and 15.94% at 40DAS, and plant dry mass by 18.97 and 15.46% at 20DAS, and 19.83 and 16.03% at 40DAS over control (Tables 24-30).

Plants grown under saline condition (50mM NaCl) also showed positive response to SA application. SA application increased the growth characteristics but the increases were lesser compared to the increases in growth characteristics of plants grown under non-saline condition (0mM NaCl).

The per cent decreases in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass due to 50mM NaCl over the control were 18.47, 18.33, 19.35, 17.74, 18.92, 18.56 and 18.97% at 20DAS, and 13.18, 13.09, 12.20, 13.33, 13.17, 13.21 and 13.22% at 40DAS in Pusa Vishal and 34.06, 33.33, 31.25, 33.77, 35.00, 34.26 and 32.99% at 20DAS, and 28.21, 28.57, 28.30, 28.76, 28.48, 29.35 and 29.17% at 40DAS in Tram, respectively.

The alleviation effects of salinity by SA were observed by comparing the per cent decrease in growth characteristics of plants under 50mM NaCl and 50mM NaCl plus 0.5mM SA in respect to control. The treatment of 0.5mM SA on plants grown with 50mM NaCl reduced the effects of 50mM NaCl. With this treatment (0.5mM SA plus 50mM NaCl), the per cent reduction in Pusa Vishal was limited to 7.21 and 0.26% for root length, 6.67 and 0.00% for root

Table 24: Effect of salicylic acid (SA) spray on root length (cm plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	22.20c	13.80b
	0.1	23.50b	14.00b
	0.5	26.40a	15.80a
	1.0	22.60c	13.70b
	NaCl (50mM)		
	SA (mM)		
	0.0	18.10f	9.10d
	0.1	18.80e	9.10d
	0.5	20.60d	10.00c
	1.0	15.50g	7.20e
	LSD at $P<0.05$	0.67	0.53
40	NaCl (0mM)		
	SA (mM)		
	0.0	38.70c	28.00b
	0.1	41.40b	28.80b
	0.5	46.40a	32.40a
	1.0	39.40c	27.80b
	NaCl (50mM)		
	SA (mM)		
	0.0	33.60e	20.10d
	0.1	35.20d	20.50d
	0.5	38.60c	22.30c
	1.0	28.50f	15.80e
	LSD at $P<0.05$	1.14	1.03

Different letters within a column indicate significant difference at $P<0.05$.

Table 25: Effect of salicylic acid (SA) spray on root fresh mass (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	1.20c	0.81b
	0.1	1.28b	0.83b
	0.5	1.43a	0.93a
	1.0	1.23c	0.81b
	NaCl (50mM)		
	SA (mM)		
	0.0	0.98e	0.54d
	0.1	1.02e	0.55d
	0.5	1.12d	0.60c
	1.0	0.84f	0.44e
	LSD at $P<0.05$	0.04	0.03
40	NaCl (0mM)		
	SA (mM)		
	0.0	2.98c	2.38
	0.1	3.19b	2.45
	0.5	3.58a	2.76
	1.0	3.04c	2.37
	NaCl (50mM)		
	SA (mM)		
	0.0	2.59e	1.70
	0.1	2.72d	1.74
	0.5	2.98c	1.89
	1.0	2.20f	1.35
	LSD at $P<0.05$	0.09	NS

Different letters within a column indicate significant difference at $P<0.05$.

Table 26: Effect of salicylic acid (SA) spray on root dry mass (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	0.31c	0.16b
	0.1	0.33b	0.16b
	0.5	0.37a	0.19a
	1.0	0.31c	0.16b
	NaCl (50mM)		
	SA (mM)		
	0.0	0.25e	0.11c
	0.1	0.26e	0.11c
	0.5	0.29d	0.12c
	1.0	0.22f	0.08d
	LSD at $P<0.05$	0.01	0.01
40	NaCl (0mM)		
	SA (mM)		
	0.0	0.82b	0.53b
	0.1	0.88b	0.54b
	0.5	0.99a	0.61a
	1.0	0.84b	0.52b
	NaCl (50mM)		
	SA (mM)		
	0.0	0.72c	0.38d
	0.1	0.75c	0.38d
	0.5	0.82b	0.42c
	1.0	0.61d	0.30e
	LSD at $P<0.05$	0.06	0.02

Different letters within a column indicate significant difference at $P<0.05$.

Table 27: Effect of salicylic acid (SA) spray on leaf fresh mass (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	1.24*c*	0.77*b*
	0.1	1.32*b*	0.78*b*
	0.5	1.48*a*	0.88*a*
	1.0	1.27*c*	0.77*b*
	NaCl (50mM)		
	SA (mM)		
	0.0	1.02*e*	0.51*d*
	0.1	1.06*e*	0.52*d*
	0.5	1.16*d*	0.56*c*
	1.0	0.87*f*	0.41*e*
	LSD at $P<0.05$	0.04	0.03
40	NaCl (0mM)		
	SA (mM)		
	0.0	7.80*c*	6.71*b*
	0.1	8.35*b*	6.91*b*
	0.5	9.36*a*	7.78*a*
	1.0	7.96*c*	6.67*b*
	NaCl (50mM)		
	SA (mM)		
	0.0	6.76*e*	4.78*d*
	0.1	7.10*d*	4.87*d*
	0.5	7.78*c*	5.30*c*
	1.0	5.75*f*	3.78*e*
	LSD at $P<0.05$	0.23	0.25

Different letters within a column indicate significant difference at $P<0.05$.

Table 28: Effect of salicylic acid (SA) spray on leaf dry mass (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	0.37c	0.20b
	0.1	0.39b	0.20b
	0.5	0.44a	0.23a
	1.0	0.37c	0.20b
	NaCl (50mM)		
	SA (mM)		
	0.0	0.30e	0.13d
	0.1	0.31e	0.13d
	0.5	0.34d	0.14c
	1.0	0.26f	0.10e
	LSD at $P<0.05$	0.01	0.01
40	NaCl (0mM)		
	SA (mM)		
	0.0	2.05c	1.58b
	0.1	2.20b	1.63b
	0.5	2.46a	1.83a
	1.0	2.09c	1.57b
	NaCl (50mM)		
	SA (mM)		
	0.0	1.78e	1.13d
	0.1	1.87d	1.15d
	0.5	2.05c	1.26c
	1.0	1.51f	0.89e
	LSD at $P<0.05$	0.06	0.06

Different letters within a column indicate significant difference at $P<0.05$.

Table 29: Effect of salicylic acid (SA) spray on leaf area (cm^2 $plant^{-1}$) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	50.10c	39.40b
	0.1	53.10b	40.10b
	0.5	59.60a	45.30a
	1.0	51.10c	39.20b
	NaCl (50mM)		
	SA (mM)		
	0.0	40.80f	25.90d
	0.1	42.40e	26.10d
	0.5	46.50d	28.40c
	1.0	35.00g	20.70e
	LSD at $P<0.05$	1.52	1.67
40	NaCl (0mM)		
	SA (mM)		
	0.0	165.80c	138.00b
	0.1	177.40b	142.10b
	0.5	198.90a	160.00a
	1.0	169.10c	137.30b
	NaCl (50mM)		
	SA (mM)		
	0.0	143.90e	97.50d
	0.1	151.00d	99.40d
	0.5	165.40c	108.20c
	1.0	122.30f	77.00e
	LSD at $P<0.05$	4.84	5.27

Different letters within a column indicate significant difference at $P<0.05$.

Table 30: Effect of salicylic acid (SA) spray on plant dry mass (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	1.16c	0.97b
	0.1	1.23b	0.99b
	0.5	1.38a	1.12a
	1.0	1.18c	0.97b
	NaCl (50mM)		
	SA (mM)		
	0.0	0.94f	0.65d
	0.1	0.98e	0.65d
	0.5	1.08d	0.71c
	1.0	0.81g	0.52e
	LSD at $P<0.05$	0.04	0.04
40	NaCl (0mM)		
	SA (mM)		
	0.0	3.63c	3.12b
	0.1	3.88b	3.22b
	0.5	4.35a	3.62a
	1.0	3.70c	3.11b
	NaCl (50mM)		
	SA (mM)		
	0.0	3.15e	2.21d
	0.1	3.30d	2.25d
	0.5	3.62c	2.45c
	1.0	2.67f	1.74e
	LSD at $P<0.05$	0.11	0.12

Different letters within a column indicate significant difference at $P<0.05$.

fresh mass, 6.45 and 0.00% for root dry mass, 6.45 and 0.26% for leaf fresh mass, 8.11 and 0.00% for leaf dry mass, 7.19 and 0.24% for leaf area and 6.90 and 0.28% for plant dry mass at 20 and 40DAS, respectively. In Tram, the decreases in the above characteristics with 0.5mM SA plus 50mM NaCl were limited to 27.54 and 20.36%, 25.93 and 20.59%, 25.00 and 20.75%, 27.27 and 21.01%, 30.00 and 20.25%, 27.92 and 21.59% and 26.80 and 21.47%, respectively at 20 and 40DAS.

4.3.1.2 Photosynthetic characteristics

Photosynthetic characteristics decreased with 50mM NaCl treatment. The effect of SA on the photosynthetic characteristics of tolerant (Pusa Vishal) and non-tolerant (Tram) cultivars was positive under non-saline and salinity stress conditions at 20 and 40DAS. The SA application also reduced the salinity stress effects on the photosynthetic characteristics of both the cultivars at both the sampling times. The concentration of 0.5mM SA was found most effective in alleviating salinity stress (Tables 31-36).

The increases in carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content in Pusa Vishal with the application of 0.5mM SA were 20.45, 18.88, 19.08, 19.20, 18.40 and 18.39%, whereas the increases in Tram were 16.38, 14.62, 15.17,15.20, 15.00 and 15.29% at 20DAS, and 19.32, 19.74, 20.10, 20.20, 19.78 and 20.00% in Pusa Vishal and 15.42, 15.43, 16.10, 16.20, 15.73 and 16.07% in Tram at 40DAS in comparison to the water-sprayed control.

The treatment of 50mM NaCl decreased carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content in Pusa Vishal and Tram. The decreases due to 50mM NaCl in these characteristics were 36.45, 13.27, 12.08, 14.30, 13.50 and 13.79% at 20DAS, and 11.30, 11.84, 10.77, 12.90, 12.09 and 12.17%, respectively at 40DAS in Pusa Vishal. A higher decreases of 43.09,

Table 31: Effect of salicylic acid (SA) spray on carbonic anhydrase activity (m mol m^{-2} leaf s^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	11.44c	9.40c
	0.1	12.26b	9.73b
	0.5	13.78a	10.94a
	1.0	11.62bc	9.38c
	NaCl (50mM)		
	SA (mM)		
	0.0	7.27e	5.35e
	0.1	7.64e	5.47e
	0.5	8.38d	5.96d
	1.0	6.16f	4.15f
	LSD at $P<0.05$	0.69	0.23
40	NaCl (0mM)		
	SA (mM)		
	0.0	15.58c	13.62b
	0.1	16.54b	14.01b
	0.5	18.59a	15.72a
	1.0	15.81b	13.57b
	NaCl (50mM)		
	SA (mM)		
	0.0	13.82d	11.04e
	0.1	14.41d	11.20d
	0.5	15.81bc	12.15c
	1.0	11.86e	8.68f
	LSD at $P<0.05$	0.82	0.73

Different letters within a column indicate significant difference at $P<0.05$.

Table 32: Effect of salicylic acid (SA) spray on net photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	19.60c	13.00b
	0.1	20.70b	13.20b
	0.5	23.30a	14.90a
	1.0	18.80d	12.10c
	NaCl (50mM)		
	SA (mM)		
	0.0	17.00f	10.00e
	0.1	17.60e	10.10e
	0.5	19.30c	11.00d
	1.0	14.60g	8.00f
	LSD at $P<0.05$	0.46	0.31
40	NaCl (0mM)		
	SA (mM)		
	0.0	22.80c	16.20b
	0.1	24.30b	16.60b
	0.5	27.30a	18.70a
	1.0	21.80d	15.10c
	NaCl (50mM)		
	SA (mM)		
	0.0	20.10f	13.20d
	0.1	21.10e	13.40d
	0.5	23.10c	14.80c
	1.0	17.00g	10.40e
	LSD at $P<0.05$	0.53	0.45

Different letters within a column indicate significant difference at $P<0.05$.

Table 33: Effect of salicylic acid (SA) spray on stomatal conductance (m mol m^{-2} s^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	414.00b	402.00b
	0.1	439.00b	410.00b
	0.5	493.00a	463.00a
	1.0	397.00d	376.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	364.00e	314.00e
	0.1	379.00e	318.00e
	0.5	413.00c	346.00d
	1.0	312.00f	251.00f
	LSD at $P<0.05$	9.45	10.23
40	NaCl (0mM)		
	SA (mM)		
	0.0	418.00c	410.00b
	0.1	448.00b	423.00b
	0.5	502.00a	476.00a
	1.0	401.00d	383.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	373.00e	339.00e
	0.1	392.00e	346.00e
	0.5	430.00b	377.00d
	1.0	316.00f	267.00f
	LSD at $P<0.05$	9.86	9.49

Different letters within a column indicate significant difference at $P<0.05$.

Table 34: Effect of salicylic acid (SA) spray on intercellular CO_2 concentration (μ mol mol^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	282.29b	280.24b
	0.1	299.79b	286.41b
	0.5	336.49a	322.84a
	1.0	271.00d	262.02c
	NaCl (50mM)		
	SA (mM)		
	0.0	241.92e	212.70e
	0.1	251.84e	215.04e
	0.5	276.03c	234.18d
	1.0	207.57f	170.00f
	LSD at $P<0.05$	6.50	7.28
40	NaCl (0mM)		
	SA (mM)		
	0.0	286.53c	284.21b
	0.1	307.16b	293.59b
	0.5	344.41a	330.25a
	1.0	275.07d	265.74c
	NaCl (50mM)		
	SA (mM)		
	0.0	249.57e	228.50e
	0.1	262.30e	233.30e
	0.5	287.26b	258.66d
	1.0	211.88f	178.00f
	LSD at $P<0.05$	6.83	7.20

Different letters within a column indicate significant difference at $P<0.05$.

Table 35: Effect of salicylic acid (SA) spray on chlorophyll content (mg g^{-1} fresh mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	1.63c	1.60b
	0.1	1.72b	1.63b
	0.5	1.93a	1.84a
	1.0	1.56e	1.49c
	NaCl (50mM)		
	SA (mM)		
	0.0	1.41g	1.23e
	0.1	1.46f	1.24e
	0.5	1.60d	1.35d
	1.0	1.21h	0.98f
	LSD at $P<0.05$	0.02	0.04
40	NaCl (0mM)		
	SA (mM)		
	0.0	1.82c	1.78b
	0.1	1.94b	1.83b
	0.5	2.18a	2.06a
	1.0	1.74d	1.66c
	NaCl (50mM)		
	SA (mM)		
	0.0	1.60e	1.45e
	0.1	1.68d	1.47d
	0.5	1.84c	1.60c
	1.0	1.36f	1.14f
	LSD at $P<0.05$	0.06	0.06

Different letters within a column indicate significant difference at $P<0.05$.

Table 36: Effect of salicylic acid (SA) spray on carotenoid content (mg g^{-1} fresh mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	0.87c	0.85b
	0.1	0.92b	0.87b
	0.5	1.03a	0.98a
	1.0	0.83d	0.79c
	NaCl (50mM)		
	SA (mM)		
	0.0	0.75f	0.65e
	0.1	0.78e	0.66e
	0.5	0.85cd	0.71d
	1.0	0.64g	0.52f
	LSD at $P<0.05$	0.02	0.02
40	NaCl (0mM)		
	SA (mM)		
	0.0	1.15c	1.12b
	0.1	1.23b	1.15b
	0.5	1.38a	1.30a
	1.0	1.10d	1.05c
	NaCl (50mM)		
	SA (mM)		
	0.0	1.01f	0.91e
	0.1	1.06e	0.93e
	0.5	1.16c	1.01d
	1.0	0.86g	0.72f
	LSD at $P<0.05$	0.03	0.03

Different letters within a column indicate significant difference at $P<0.05$.

23.08, 21.89, 24.10, 23.13 and 23.53% in the above characteristics, however, were observed in Tram at 20DAS, and 18.94, 18.52, 17.32, 19.60, 18.54 and 18.75% at 40DAS.

The decreases in carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content due to 50mM NaCl were reduced with the application of 0.5mM SA given to NaCl-treated plants. The decreases in these characteristics were limited to 26.75, 1.53, 0.24, 2.22, 1.84 and 2.30% in Pusa Vishal and 36.60, 15.38, 13.93, 16.44, 15.63 and 16.47% in Tram at 20DAS, and 10.79, 8.64, 8.05, 8.99, 10.11 and 9.82% in Tram at 40DAS, and 10.79, 8.64, 8.05, 8.99, 10.11 and 9.82% in Tram at 40DAS due to the treatment 0.5mM SA plus 50mM NaCl compared to 50mM NaCl.

At 40DAS, the effect of 0.5mM SA on salinity-treated plants was also significant. The treatment of 0.5mM SA not only nullified the adverse effects of 50mM NaCl but also increased the characteristics in comparison to the respective control (Tables 31-36).

4.3.1.3 Biochemical characteristics

SA significantly ameliorated the salinity effects on biochemical characteristics. SA application decreased the concentrations of sodium and chloride in both tolerant (Pusa Vishal) and non-tolerant (Tram) cultivars, and the effect of 0.5mM SA was found more pronounced than the other SA concentrations under normal and saline conditions at both the sampling times (Tables 37-38).

SA application significantly enhanced nitrogen, phosphorus. potassium and calcium concentrations under both normal and saline conditions in both the cultivars, but the effect was comparatively more pronounced with 0.5mM SA. (Tables 39-42).

The decrease in sodium and chloride concentrations with 0.5mM SA application was 30.93 and 30.65% in Pusa Vishal at 20DAS, and 35.14 and

35.00% at 40DAS with respect to control. The cultivar Tram showed lesser decrease with 0.5mM SA, which was 25.23 and 25.64% at 20DAS, and 17.86 and 18.32% at 40DAS compared to the control.

SA application on plants treated with 50mM NaCl significantly reduced the concentrations of sodium and chloride in Pusa Vishal and Tram in comparison to the concentrations noted in 50mM NaCl treatment. In 50mM NaCl treatment, the concentrations of sodium and chloride were increased to 6.19 and 12.9% at 20DAS, and 5.41, 5.83% at 40DAS in Pusa Vishal and 9.91 and 16.67% at 20DAS, and 8.33 and 14.50% at 40DAS in Tram compared to 0mM NaCl. However, with the application of 0.5mM SA, the accumulation of sodium and chloride restricted to 30.93 and 27.42% at 20DAS, and 32.43 and 32.50% at 40DAS in Pusa Vishal, and 23.42 and 19.23% at 20DAS, and 17.26 and 12.21% at 40DAS in Tram.

Under no salinity stress, the application of 0.5mM SA on Pusa Vishal increased nitrogen by 32.72 and 31.05%, phosphorus by 32.91 and 30.61%, potassium by 32.26 and 31.11% and calcium by 32.77 and 31.11%, respectively at 20 and 40DAS in comparison to the control. Similarly, in Tram the above characteristics were increased by 29.15 and 28.18%, 29.17 and 28.24%, 28.75 and 28.40% and 29.09 and 28.24% at 20 and 40DAS, respectively.

In contrast, treatment of 50mM NaCl reduced nitrogen, phosphorus, potassium and calcium concentrations by 20.80, 41.77, 8.60 and 15.97% and 13.72, 29.59, 2.78 and 12.78% at 20 and 40DAS in Pusa Vishal. The above characteristics in Tram were decreased by 32.66, 56.94, 8.75 and 19.09% and 16.36, 38.82, 6.51 and 16.47% at 20 and 40DAS, respectively.

In Pusa Vishal, the nitrogen concentration increased with the combined application of 0.5mM SA and 50mM NaCl in comparison to control at 20 and 40DAS. In Tram, the decrease in the above characteristic was restricted to 17.09 and 4.55% at 20 and 40DAS, respectively. In Pusa Vishal, the decrease

Table 37: Effect of salicylic acid (SA) spray on sodium concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	9.70b	11.10b
	0.1	7.70d	9.10e
	0.5	6.70e	8.30g
	1.0	8.90c	10.60c
	NaCl (50mM)		
	SA (mM)		
	0.0	10.30a	12.20a
	0.1	7.60d	9.30d
	0.5	6.70e	8.50f
	1.0	9.00bc	11.20b
	LSD at $P<0.05$	0.14	0.19
40	NaCl (0mM)		
	SA (mM)		
	0.0	14.80b	16.80c
	0.1	11.70e	14.10d
	0.5	9.60h	13.80e
	1.0	12.60d	16.50c
	NaCl (50mM)		
	SA (mM)		
	0.0	15.60a	18.20a
	0.1	11.00f	14.20d
	0.5	10.00g	13.90de
	1.0	13.60c	17.40b
	LSD at $P<0.05$	0.21	0.31

Different letters within a column indicate significant difference at $P<0.05$.

Table 38: Effect of salicylic acid (SA) spray on chloride concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	6.20b	7.80c
	0.1	4.90e	6.40f
	0.5	4.30g	5.80g
	1.0	5.70c	7.50d
	NaCl (50mM)		
	SA (mM)		
	0.0	7.00a	9.10a
	0.1	5.20d	7.00e
	0.5	4.50f	6.30f
	1.0	6.10b	8.30b
	LSD at $P<0.05$	0.16	0.20
40	NaCl (0mM)		
	SA (mM)		
	0.0	12.00b	13.10c
	0.1	8.70f	11.00f
	0.5	7.80h	10.70g
	1.0	10.20d	12.80d
	NaCl (50mM)		
	SA (mM)		
	0.0	12.70a	15.00a
	0.1	9.00e	11.70e
	0.5	8.10g	11.50e
	1.0	11.10c	14.30b
	LSD at $P<0.05$	0.20	0.23

Different letters within a column indicate significant difference at $P<0.05$.

Table 39: Effect of salicylic acid (SA) spray on nitrogen concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	32.70d	19.90c
	0.1	38.80b	21.80b
	0.5	43.40a	25.70a
	1.0	34.00c	19.40c
	NaCl (50mM)		
	SA (mM)		
	0.0	25.90f	13.40f
	0.1	28.80e	14.60e
	0.5	34.00c	16.50d
	1.0	25.10f	13.00f
	LSD at $P<0.05$	0.82	0.53
40	NaCl (0mM)		
	SA (mM)		
	0.0	55.40d	44.00c
	0.1	60.40b	46.50b
	0.5	72.60a	56.40a
	1.0	56.90c	43.20c
	NaCl (50mM)		
	SA (mM)		
	0.0	47.80f	36.80f
	0.1	51.70e	38.80e
	0.5	56.30cd	42.00d
	1.0	46.60g	35.80g
	LSD at $P<0.05$	1.19	0.90

Different letters within a column indicate significant difference at $P<0.05$.

Table 40: Effect of salicylic acid (SA) spray on phosphorus concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	7.90d	7.20c
	0.1	9.40b	7.90b
	0.5	10.50a	9.30a
	1.0	8.20c	7.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	4.60g	3.10f
	0.1	5.10f	3.40e
	0.5	6.00e	3.80d
	1.0	4.50g	3.00f
	LSD at $P<0.05$	0.24	0.29
40	NaCl (0mM)		
	SA (mM)		
	0.0	9.80d	8.50c
	0.1	10.70b	9.00b
	0.5	12.80a	10.90a
	1.0	10.10c	8.30c
	NaCl (50mM)		
	SA (mM)		
	0.0	6.90g	5.20f
	0.1	7.40f	5.50e
	0.5	8.10e	5.90d
	1.0	6.70h	5.10f
	LSD at $P<0.05$	0.23	0.24

Different letters within a column indicate significant difference at $P<0.05$.

Table 41: Effect of salicylic acid (SA) spray on potassium concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	9.30e	8.00d
	0.1	11.00b	8.80c
	0.5	12.30a	10.30a
	1.0	9.70c	7.80e
	NaCl (50mM)		
	SA (mM)		
	0.0	8.50f	7.30f
	0.1	9.50d	8.00d
	0.5	11.10b	9.00b
	1.0	8.20g	7.00g
	LSD at $P<0.05$	0.19	0.17
40	NaCl (0mM)		
	SA (mM)		
	0.0	18.00f	16.90c
	0.1	19.60c	17.90b
	0.5	23.60a	21.70a
	1.0	18.50e	16.60c
	NaCl (50mM)		
	SA (mM)		
	0.0	17.50g	15.80d
	0.1	18.90d	16.70c
	0.5	20.60b	18.00b
	1.0	17.10h	15.40e
	LSD at $P<0.05$	0.37	0.36

Different letters within a column indicate significant difference at $P<0.05$.

Table 42: Effect of salicylic acid (SA) spray on calcium concentration (mg g^{-1} dry mass) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 and 40 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
20	NaCl (0mM)		
	SA (mM)		
	0.0	11.90e	11.00c
	0.1	14.10b	12.00b
	0.5	15.80a	14.20a
	1.0	12.40d	10.70d
	NaCl (50mM)		
	SA (mM)		
	0.0	10.00g	8.90f
	0.1	11.10f	9.70e
	0.5	13.10c	11.00c
	1.0	9.70h	8.60g
	LSD at $P<0.05$	0.26	0.23
40	NaCl (0mM)		
	SA (mM)		
	0.0	18.00d	17.00c
	0.1	19.60b	18.00b
	0.5	23.60a	21.80a
	1.0	18.50c	16.70c
	NaCl (50mM)		
	SA (mM)		
	0.0	15.70f	14.20f
	0.1	17.00e	15.00e
	0.5	18.50c	16.20d
	1.0	15.30g	13.80g
	LSD at $P<0.05$	0.38	0.39

Different letters within a column indicate significant difference at $P<0.05$.

in phosphorus was restricted to 24.05% with 0.5mM SA plus 50mM NaCl at 20DAS, and 17.35% at 40DAS. In Tram, the above characteristic was decreased by 47.22% at 20DAS, and 30.59% respectively at 40DAS.

Application of 0.5mM SA treatment had significant effect in restricting the accumulation of potassium and calcium concentrations compared with control (0mM NaCl) and salt treatment (50mM NaCl). In Pusa Vishal, potassium concentration increased by 19.35 and 14.44% with 0.5mM SA plus 50mM NaCl at 20 and 40DAS. In Tram, it was increased by 12.50 and 6.51% at 20 and 40DAS. Application of 0.5mM SA plus 50mM NaCl on Pusa Vishal increased calcium concentration by 10.08 and 2.78% at 20 and 40DAS, respectively in comparison to control. However, in Tram, the SA application restored the decrease to the level of control.

4.3.1.4 Activities of antioxidative enzymes

The effect of SA on the activities of antioxidative enzymes was studied under normal and saline conditions at initial stage (20DAS) of growth. SA enhanced the activities of antioxidative enzymes significantly. At 50mM NaCl, both cultivars Pusa Vishal and Tram showed an increase in the activities of antioxidative enzymes (Tables 43-44).

Under non-saline condition, the activities of antioxidative enzymes in both the cultivars increased significantly with 0.5mM SA application. With the application of 0.5mM SA, catalase activity of Pusa Vishal and Tram increased by 34.40 and 31.47%, superoxide dismutase activity by 34.50 and 31.78%, glutathione reductase activity by 34.78 and 31.66% and ascorbate peroxidase activity by 35.16 and 31.25%, respectively.

Activities of antioxidative enzymes under salinized (50mM NaCl) condition were also increased by SA application and were greater in salinized condition (50mM NaCl). Catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase activities of Pusa Vishal were increased by 11.72, 7.69, 8.70 and 12.50% in response to 50mM NaCl treatment. The above

Table 43: Effect of salicylic acid (SA) spray on catalase activity (U g^{-1} FM min^{-1}) and superoxide dismutase activity (U mg^{-1} protein) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
		Catalase	
20	NaCl (0mM)		
	SA (mM)		
	0.0	145.00g	136.00g
	0.1	182.12d	164.02d
	0.5	194.88c	178.80c
	1.0	126.90h	125.10h
	NaCl (50mM)		
	SA (mM)		
	0.0	162.00e	158.00e
	0.1	199.75b	187.39b
	0.5	211.57a	198.13a
	1.0	149.70f	151.00f
	LSD at $P<0.05$	3.89	3.43
		Superoxide dismutase	
20	NaCl (0mM)		
	SA (mM)		
	0.0	26.00e	18.00f
	0.1	32.66c	21.73c
	0.5	34.97b	23.72b
	1.0	22.78f	16.42g
	NaCl (50mM)		
	SA (mM)		
	0.0	28.00d	20.00d
	0.1	34.52b	23.72b
	0.5	36.51a	25.12a
	1.0	25.87e	19.06e
	LSD at $P<0.05$	0.66	0.45

Different letters within a column indicate significant difference at $P<0.05$.

Table 44: Effect of salicylic acid (SA) spray on glutathione reductase activity (U mg^{-1} protein) and ascorbate peroxidase activity (U mg^{-1} protein) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at 20 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
		Glutathione reductase	
20	NaCl (0mM)		
	SA (mM)		
	0.0	0.230e	0.199e
	0.1	0.289c	0.240c
	0.5	0.310b	0.262a
	1.0	0.201f	0.182f
	NaCl (50mM)		
	SA (mM)		
	0.0	0.250d	0.204d
	0.1	0.309b	0.242c
	0.5	0.326a	0.256b
	1.0	0.232e	0.195e
	LSD at $P<0.05$	0.006	0.004
		Ascorbate peroxidase	
20	NaCl (0mM)		
	SA (mM)		
	0.0	1.28g	0.96f
	0.1	1.61d	1.16c
	0.5	1.73c	1.26b
	1.0	1.12h	0.88g
	NaCl (50mM)		
	SA (mM)		
	0.0	1.44e	1.06d
	0.1	1.78b	1.26b
	0.5	1.88a	1.33a
	1.0	1.33f	1.01e
	LSD at $P<0.05$	0.034	0.024

Different letters within a column indicate significant difference at $P<0.05$.

characteristics of Tram were increased by 16.18, 11.11, 2.51 and 10.42% respectively, in response to 50 mM NaCl. Application of 0.5mM SA on plants treated with 50mM NaCl exhibited higher increase in the activities of antioxidative enzymes compared with the increase observed in 50mM NaCl treatment. The increase in catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase activities of Pusa Vishal and Tram was 45.91, 40.42, 41.74 and 46.88% and 45.68, 39.56, 28.64 and 38.54% respectively due to 50mM NaCl plus 0.5mM SA in comparison to control.

4.3.1.5 Yield characteristics

Yield characteristics decreased significantly with 50mM NaCl in both the cultivars, but more adverse effects of salinity were found on Tram. Application of 0.5mM SA treatment had a significant effect in reducing the effect of salinity on yield characteristics (Tables 45-46).

The application of 0.5mM SA on Pusa Vishal and Tram grown under 0mM NaCl (control) increased pod length by 19.88 and 15.89%, pod number by 19.94 and 15.83%, seed number by 20.17 and 15.60% and seed yield by 20.09 and 15.80% in comparison to the control.

At 50mM NaCl, the per cent decrease in pod length, pod number, seed number and seed yield over control in Pusa Vishal was 13.30, 13.39, 13.45 and 13.54%, respectively. In Tram, a decrease of 28.43, 29.34, 29.36 and 28.43% in the above characteristics was recorded with 50mM NaCl.

At 50mM NaCl plus 0.5mM SA treatment, the reductions were less compared to 50mM NaCl treatment alone. It was 0.41 and 20.57% in pod length, 0.57 and 21.62% in pod number, 0.84 and 22.02% in seed number and 0.56 and 20.47% in seed yield in Pusa Vishal and Tram, respectively (Tables 45-46).

Table 45: Effect of salicylic acid (SA) spray on pod length (cm) and pod number plant^{-1} of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at harvest, i.e., 60 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
		Pod length	
60	NaCl (0mM)		
	SA (mM)		
	0.0	6.69c	5.98b
	0.1	7.16b	6.15b
	0.5	8.02a	6.93a
	1.0	6.82c	5.95b
	NaCl (50mM)		
	SA (mM)		
	0.0	5.80e	4.28d
	0.1	6.08d	4.36d
	0.5	6.66c	4.75c
	1.0	4.93f	3.38e
	LSD at $P<0.05$	0.19	0.22
		Pod number	
60	NaCl (0mM)		
	SA (mM)		
	0.0	35.10c	25.90bc
	0.1	37.20b	26.60b
	0.5	42.10a	30.00a
	1.0	35.80c	25.80c
	NaCl (50mM)		
	SA (mM)		
	0.0	30.40e	18.30e
	0.1	31.90d	18.70e
	0.5	34.90c	20.30d
	1.0	25.80f	14.40f
	LSD at $P<0.05$	1.02	0.98

Different letters within a column indicate significant difference at $P<0.05$.

Table 46: Effect of salicylic acid (SA) spray on seed number pod^{-1} and seed yield (g plant^{-1}) of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) grown under salinity stress at harvest, i.e., 60 days after sowing (DAS).

DAS	Treatments	Pusa Vishal	Tram
		Seed number	
60	NaCl (0mM)		
	SA (mM)		
	0.0	11.90bc	10.90b
	0.1	12.70b	11.20b
	0.5	14.30a	12.60a
	1.0	12.10b	10.80b
	NaCl (50mM)		
	SA (mM)		
	0.0	10.30d	7.70d
	0.1	10.80d	7.80d
	0.5	11.80c	8.50c
	1.0	8.80e	6.00e
	LSD at $P<0.05$	0.82	0.41
		Seed yield	
60	NaCl (0mM)		
	SA (mM)		
	0.0	8.86c	7.28b
	0.1	9.48b	7.50b
	0.5	10.64a	8.43a
	1.0	9.04c	7.24b
	NaCl (50mM)		
	SA (mM)		
	0.0	7.66e	5.21d
	0.1	8.04d	5.31d
	0.5	8.81c	5.79c
	1.0	6.51f	4.12e
	LSD at $P<0.05$	0.31	0.31

Different letters within a column indicate significant difference at $P<0.05$.

4.3.2 Experiment 4

4.3.2.1 Growth characteristics

The effect of the application of SA was found significant on growth characteristics at all sampling times. The application of SA increased the growth characteristics and the increases were greater under non-saline (control) condition than under saline condition. Application of 0.5mM SA helped to reduce the adverse effects of salinity on growth characteristics (Tables 47-53).

Growth characteristics increased significantly over control with 0.5mM SA application under non-saline condition. In Alankar, the increase in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass with 0.5mM SA was 18.63, 18.90, 19.10, 19.02, 18.84, 18.96 and 19.13% at 30DAS; 19.87, 20.00, 19.88, 20.01, 20.04, 20.00 and 19.99% at 60DAS, and 20.91, 21.01, 20.87, 21.02, 21.02, 20.92 and 20.96% respectively at 90DAS. In PBM16, the root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass increased by 14.78, 15.00, 15.38, 15.01, 15.19, 14.96 and 15.09% due to 0.5mM SA at 30DAS; 15.98, 16.07, 15.89, 15.98, 16.20, 15.93 and 15.96% at 60DAS, and 16.93, 17.00, 16.78, 16.97, 16.94, 16.92 and 17.02% respectively at 90DAS.

Comparing the salinity effects, it was found that it decreased growth characteristics substantially. In Alankar, the reduction in root length was 23.57, 18.27 and 13.46%, root fresh mass was 23.41, 18.23 and 13.13%, root dry mass was 23.03, 18.44 and 13.58%, leaf fresh mass was 23.56, 18.49 and 13.47%, leaf dry mass was 23.55, 18.26 and 13.43%, leaf area was 23.26, 18.40 and 13.23% and plant dry mass was 23.47, 18.52 and 13.27% at 30, 60 and 90DAS, respectively due to 50mM NaCl. In the cultivar PBM16, a higher decrease in growth was observed. Regarding per cent decrease in PBM16 due to 50mM NaCl, it exhibited 38.26, 39.55, 40.00, 39.48, 39.24, 39.53 and 39.55% decreases in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass, respectively at 30DAS. The decreases

in these characteristics at 60DAS were 33.61, 34.42, 33.18, 33.57, 33.10, 34.45 and 33.96% and 28.84, 29.33, 28.95, 29.22, 28.83, 28.85 and 29.28%, respectively at 90DAS (Tables 47-53).

Application of SA alleviated the NaCl stress effects. Under non-saline condition, 0.5mM SA application significantly enhanced growth characteristics. In Alankar, the treatment of 0.5mM SA on plants grown with 50mM NaCl resulted in a lesser decrease than the treatment 50mM NaCl alone. The decrease was 12.93% in root length, 12.54% in root fresh mass, 12.36% in root dry mass, 12.80% in leaf fresh mass, 13.04% in leaf dry mass, 12.52% in leaf area and 12.70% in plant dry mass at 30DAS in comparison to control. At 60DAS, the decreases in the above characteristics were 6.09%, 5.95%, 6.34%, 6.24%, 1.95%, 6.27% and 6.34%, respectively in plants receiving 50mM NaCl plus 0.5mM SA treatment in comparison to control. At 90DAS, the application of 0.5mM SA proved much more beneficial under saline condition. It not only reduced the per cent reduction due to 50mM NaCl but even increased the characteristics in comparison to the respective control. The increases were 0.24%, 0.72%, 0.20%, 0.38%, 0.37%, 0.66% and 0.59% in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass, respectively compared to control. The other cultivar, PBM16 also responded similarly to Alankar with respect to 50mM NaCl plus 0.5mM SA treatment and reduced the adverse effects of salinity. The decrease in root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass in PBM16 with 50 mM NaCl plus 0.5 mM SA treatment were only 32.17, 33.64, 33.85, 33.44, 33.54, 33.55 and 33.15% at 30DAS; 26.64, 27.17, 25.70, 26.30, 25.93, 27.31 and 26.66% at 60DAS, and 20.37, 20.94, 20.39, 20.74, 20.36, 20.39 and 20.75% respectively at 90DAS.

The treatment 50mM NaCl plus 0.5mM SA could not enhance the growth characteristics at 90DAS in PBM16 as was observed in Alankar.

Table 47: Effect of salicylic acid (SA) spray on root length (cm plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	26.30c	23.00b
	0.1	27.80b	23.40b
	0.5	31.20a	26.40a
	1.0	26.80c	22.80b
	NaCl (50mM)		
	SA (mM)		
	0.0	20.10e	14.20d
	0.1	20.90e	14.30d
	0.5	22.90d	15.60c
	1.0	17.20f	11.30e
	LSD at $P<0.05$	0.94	1.00
60	NaCl (0mM)		
	SA (mM)		
	0.0	31.20c	24.40b
	0.1	33.30b	25.10b
	0.5	37.40a	28.30a
	1.0	31.80c	24.20b
	NaCl (50mM)		
	SA (mM)		
	0.0	25.50f	16.20d
	0.1	26.70e	16.50d
	0.5	29.30d	17.90c
	1.0	21.60g	12.70e
	LSD at $P<0.05$	0.98	1.02
90	NaCl (0mM)		
	SA (mM)		
	0.0	41.60c	37.80c
	0.1	44.90b	39.30b
	0.5	50.30a	44.20a
	1.0	40.70c	37.60d
	NaCl (50mM)		
	SA (mM)		
	0.0	36.00e	26.90f
	0.1	38.10d	27.70f
	0.5	41.70c	30.10e
	1.0	30.20f	20.90g
	LSD at $P<0.05$	1.20	1.48

Different letters within a column indicate significant difference at $P<0.05$.

Table 48: Effect of salicylic acid (SA) spray on root fresh mass (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	5.98c	2.20b
	0.1	6.33b	2.24b
	0.5	7.11a	2.53a
	1.0	6.10c	2.19b
	NaCl (50mM)		
	SA (mM)		
	0.0	4.58e	1.33d
	0.1	4.77e	1.34d
	0.5	5.23d	1.46c
	1.0	3.94f	1.06e
	LSD at $P<0.05$	0.20	0.10
60	NaCl (0mM)		
	SA (mM)		
	0.0	10.75c	7.03b
	0.1	11.50b	7.24b
	0.5	12.90a	8.16a
	1.0	10.96c	7.00b
	NaCl (50mM)		
	SA (mM)		
	0.0	8.79f	4.61d
	0.1	9.23e	4.71d
	0.5	10.11d	5.12c
	1.0	7.47g	3.65e
	LSD at $P<0.05$	0.34	0.29
90	NaCl (0mM)		
	SA (mM)		
	0.0	15.23c	9.41c
	0.1	16.45b	9.79b
	0.5	18.43a	11.01a
	1.0	15.53c	9.36c
	NaCl (50mM)		
	SA (mM)		
	0.0	13.23e	6.65e
	0.1	14.02d	6.85e
	0.5	15.34c	7.44d
	1.0	11.11f	5.18f
	LSD at $P<0.05$	0.46	0.36

Different letters within a column indicate significant difference at $P<0.05$.

Table 49: Effect of salicylic acid (SA) spray on root dry mass (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	1.78c	0.65b
	0.1	1.89b	0.66b
	0.5	2.12a	0.75a
	1.0	1.82c	0.64b
	NaCl (50mM)		
	SA (mM)		
	0.0	1.37e	0.39d
	0.1	1.42e	0.40cd
	0.5	1.56d	0.43c
	1.0	1.18f	0.31e
	LSD at $P<0.05$	0.06	0.03
60	NaCl (0mM)		
	SA (mM)		
	0.0	3.47c	2.14b
	0.1	3.71b	2.21b
	0.5	4.16a	2.48a
	1.0	3.54c	2.13b
	NaCl (50mM)		
	SA (mM)		
	0.0	2.83f	1.43d
	0.1	2.97e	1.46d
	0.5	3.25d	1.59c
	1.0	2.40g	1.13e
	LSD at $P<0.05$	0.11	0.09
90	NaCl (0mM)		
	SA (mM)		
	0.0	5.08c	3.04bc
	0.1	5.48b	3.16b
	0.5	6.14a	3.55a
	1.0	5.18c	3.02c
	NaCl (50mM)		
	SA (mM)		
	0.0	4.39e	2.16e
	0.1	4.65d	2.22e
	0.5	5.09c	2.42d
	1.0	3.69f	1.68f
	LSD at $P<0.05$	0.15	0.12

Different letters within a column indicate significant difference at $P<0.05$.

Table 50: Effect of salicylic acid (SA) spray on leaf fresh mass (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	10.78c	6.13b
	0.1	11.43b	6.25b
	0.5	12.83a	7.05a
	1.0	11.00c	6.10b
	NaCl (50mM)		
	SA (mM)		
	0.0	8.24e	3.71d
	0.1	8.57e	3.75d
	0.5	9.40d	4.08c
	1.0	7.09f	2.97e
	LSD at $P<0.05$	0.37	0.28
60	NaCl (0mM)		
	SA (mM)		
	0.0	20.34c	16.77b
	0.1	21.77b	17.27b
	0.5	24.41a	19.45a
	1.0	20.75c	16.69b
	NaCl (50mM)		
	SA (mM)		
	0.0	16.58f	11.14d
	0.1	17.41e	11.36d
	0.5	19.07d	12.36c
	1.0	14.09g	8.80e
	LSD at $P<0.05$	0.64	0.69
90	NaCl (0mM)		
	SA (mM)		
	0.0	25.98c	22.28bc
	0.1	28.06b	23.17b
	0.5	31.44a	26.06a
	1.0	26.50c	22.17c
	NaCl (50mM)		
	SA (mM)		
	0.0	22.48e	15.77e
	0.1	23.83d	16.24e
	0.5	26.08c	17.66d
	1.0	18.88f	12.30f
	LSD at $P<0.05$	0.78	0.86

Different letters within a column indicate significant difference at $P<0.05$.

Table 51: Effect of salicylic acid (SA) spray on leaf dry mass (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	2.76c	1.58b
	0.1	2.92b	1.61b
	0.5	3.28a	1.82a
	1.0	2.81c	1.57b
	NaCl (50mM)		
	SA (mM)		
	0.0	2.11e	0.96d
	0.1	2.19e	0.97d
	0.5	2.40d	1.05c
	1.0	1.81f	0.77e
	LSD at $P<0.05$	0.09	0.07
60	NaCl (0mM)		
	SA (mM)		
	0.0	5.64cd	4.32b
	0.1	6.03b	4.45b
	0.5	6.77a	5.02a
	1.0	5.75c	4.30b
	NaCl (50mM)		
	SA (mM)		
	0.0	4.61f	2.89d
	0.1	4.84e	2.94d
	0.5	5.53d	3.20c
	1.0	3.92g	2.28e
	LSD at $P<0.05$	0.18	0.18
90	NaCl (0mM)		
	SA (mM)		
	0.0	8.04c	6.14c
	0.1	8.69b	6.38b
	0.5	9.73a	7.18a
	1.0	8.20c	6.11c
	NaCl (50mM)		
	SA (mM)		
	0.0	6.96e	4.37e
	0.1	7.38d	4.50e
	0.5	8.07c	4.89d
	1.0	5.84f	3.41f
	LSD at $P<0.05$	0.24	0.23

Different letters within a column indicate significant difference at $P<0.05$.

Table 52: Effect of salicylic acid (SA) spray on leaf area (cm^2 $plant^{-1}$) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	559.00c	468.00b
	0.1	592.00b	477.00b
	0.5	665.00a	538.00a
	1.0	570.00c	465.00b
	NaCl (50mM)		
	SA (mM)		
	0.0	429.00e	283.00d
	0.1	446.00e	285.00d
	0.5	489.00d	311.00c
	1.0	368.00f	226.00e
	LSD at $P<0.05$	18.88	21.07
60	NaCl (0mM)		
	SA (mM)		
	0.0	750.00bc	659.00b
	0.1	802.00b	678.00b
	0.5	900.00a	764.00a
	1.0	765.00b	655.00b
	NaCl (50mM)		
	SA (mM)		
	0.0	612.00d	432.00d
	0.1	642.00d	440.00d
	0.5	703.00c	479.00c
	1.0	520.00e	341.00e
	LSD at $P<0.05$	53.22	27.21
90	NaCl (0mM)		
	SA (mM)		
	0.0	1066.00c	863.00b
	0.1	1151.00b	897.00b
	0.5	1289.00a	1,009.00a
	1.0	1087.00c	858.00b
	NaCl (50mM)		
	SA (mM)		
	0.0	925.00e	614.00d
	0.1	980.00d	632.00cd
	0.5	1073.00c	687.00c
	1.0	777.00f	478.00e
	LSD at $P<0.05$	32.02	62.97

Different letters within a column indicate significant difference at $P<0.05$.

Table 53: Effect of salicylic acid (SA) spray on plant dry mass (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	6.22c	3.71b
	0.1	6.60b	3.79b
	0.5	7.41a	4.27a
	1.0	6.35c	3.69b
	NaCl (50mM)		
	SA (mM)		
	0.0	4.76e	2.25d
	0.1	4.95e	2.27d
	0.5	5.43d	2.48c
	1.0	4.09f	1.80e
	LSD at $P<0.05$	0.21	0.17
60	NaCl (0mM)		
	SA (mM)		
	0.0	14.36c	11.78b
	0.1	15.37b	12.13b
	0.5	17.23a	13.66a
	1.0	14.65c	11.72b
	NaCl (50mM)		
	SA (mM)		
	0.0	11.70f	7.78d
	0.1	12.28e	7.94d
	0.5	13.45d	8.64c
	1.0	9.94g	6.15f
	LSD at $P<0.05$	0.46	0.49
90	NaCl (0mM)		
	SA (mM)		
	0.0	21.85c	19.57c
	0.1	23.59b	20.36b
	0.5	26.43a	22.90a
	1.0	22.28bc	19.48c
	NaCl (50mM)		
	SA (mM)		
	0.0	18.95e	13.84e
	0.1	20.09d	14.26e
	0.5	21.98c	15.51d
	1.0	15.92f	10.80f
	LSD at $P<0.05$	0.66	0.75

Different letters within a column indicate significant difference at $P<0.05$.

4.3.2.2 Photosynthetic characteristics

Photosynthetic characteristics of both the cultivars decreased with 50mM NaCl. The application of 0.5mM SA increased the characteristics of plants treated with 0mM NaCl (control) and also of those plants treated with 50mM NaCl (Tables 54-59). The treatment of 0.5mM SA also reduced the adverse effects of 50mM NaCl.

Photosynthetic characteristics increased markedly due to the application of 0.5mM SA under non-saline condition. The increases in carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content in Alankar with 0.5mM SA were 20.09, 18.78, 19.04, 19.20, 18.97 and 19.35% at 30DAS; 19.79, 19.72, 16.76, 20.20, 20.00 and 19.69% at 60DAS, and 18.27, 20.69, 21.22, 21.20, 20.55 and 20.90% at 90DAS, respectively. In PBM16, these increases were lesser. Carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content increased by 16.22, 15.30, 15.00, 15.20, 14.71 and 15.38% due to 0.5mM SA at 30DAS; 15.21, 15.69, 16.01, 16.20, 15.63 and 15.70% at 60DAS, and 15.29, 16.84, 17.17, 17.20, 16.91 and 16.84% at 90DAS.

Photosynthetic characteristics decreased significantly with 50mM NaCl in both the cultivars but more adverse effects of salinity were found on PBM16. The treatment of 50mM NaCl on Alankar and PBM16 decreased carbonic anhydrase activity by 17.85 and 25.63% at 30DAS; 11.06 and 19.87% at 60DAS, and 9.99 and 18.93% at 90DAS; net photosynthetic rate by 13.20 and 22.95% at 30DAS; 11.93 and 18.63% at 60DAS, and 11.33 and 16.32% at 90DAS; stomatal conductance by 12.29 and 21.75% at 30DAS; 10.77 and 17.49% at 60DAS, and 10.24 and 15.15% at 90DAS; intercellular CO_2 concentration by 14.30 and 23.10% at 30DAS; 13.00 and 19.70% at 60DAS, and 12.40 and 17.40% at 90DAS; chlorophyll content by 13.22 and 22.94% at 30DAS; 12.00 and 18.75% at 60DAS, and 11.42 and 16.43% at 90DAS, and

carotenoid content by 13.98 and 23.08% at 30DAS; 11.81 and 18.18% at 60DAS, and 11.44 and 16.32% at 90DAS over control.

The application of SA showed variable ability to alleviate the NaCl inhibitory effects in the two cultivars. In the cultivar Alankar, the application of 0.5mM SA on plants treated with 50mM NaCl alleviated the adverse effects of 50mM NaCl alone, and the extent of reduction was lesser compared with the combined application of SA and NaCl. Carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, chlorophyll content and carotenoid content in Alankar were decreased by 5.40, 1.52, 0.24, 2.21, 1.15 and 2.15%, respectively at 30DAS. However, at later growth stages (60 and 90DAS) the treatment of 0.5mM SA not only reduced the adverse effects of NaCl but overcome the effect and increased the characteristics. Carbonic anhydrase activity was increased by 2.10 and 2.35%, net photosynthetic rate by 0.92 and 2.46%, stomatal conductance by 2.81 and 4.39%, intercellular CO_2 concentration by 0.14 and 1.70%, chlorophyll content by 1.00 and 2.74% and carotenoid content by 1.57 and 2.49% at these two stages in Alankar. In PBM16, the application of 0.5mM SA only reduced the adverse effects of 50mM NaCl at all the growth stages. The per cent reduction in carbonic anhydrase activity was 16.97, 11.40 and 10.92, in net photosynthetic rate was 15.30, 9.80 and 6.32, in stomatal conductance was 13.75, 8.13 and 4.80, in intercellular CO_2 concentration was 15.33, 10.79 and 7.40, in chlorophyll content was 15.29, 9.90 and 6.76 and in carotenoid content was 15.38, 9.09 and 6.32 at 30, 60 and 90DAS, respectively.

4.3.2.3 Biochemical characteristics

Application of SA proved effective in ameliorating the adverse effects of salinity on biochemical characteristics.

The effects of SA application on sodium and chloride concentrations were found significant at all stages, except for sodium concentration at 60DAS in Alankar (Tables 60-61). The concentrations of sodium and chloride

Table 54: Effect of salicylic acid (SA) spray on carbonic anhydrase activity (m mol m^{-2} leaf s^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	13.89c	11.90c
	0.1	14.94b	12.30b
	0.5	16.68a	13.83a
	1.0	14.08c	11.85c
	NaCl (50mM)		
	SA (mM)		
	0.0	11.41f	8.85e
	0.1	12.03e	9.10e
	0.5	13.14d	9.88d
	1.0	9.62g	6.87f
	LSD at $P<0.05$	0.26	0.30
60	NaCl (0mM)		
	SA (mM)		
	0.0	18.09e	16.31c
	0.1	19.21b	16.66b
	0.5	21.67a	18.79a
	1.0	18.38d	16.26c
	NaCl (50mM)		
	SA (mM)		
	0.0	16.09g	13.07f
	0.1	16.83f	13.25e
	0.5	18.47c	14.45d
	1.0	13.79h	10.23g
	LSD at $P<0.05$	0.33	0.30
90	NaCl (0mM)		
	SA (mM)		
	0.0	17.02e	15.11bc
	0.1	17.90b	15.34b
	0.5	20.13a	17.42a
	1.0	17.33d	15.05c
	NaCl (50mM)		
	SA (mM)		
	0.0	15.32g	12.25e
	0.1	15.82f	12.31e
	0.5	17.42c	13.46d
	1.0	13.25h	9.75f
	LSD at $P<0.05$	0.29	0.27

Different letters within a column indicate significant difference at $P<0.05$.

Table 55: Effect of salicylic acid (SA) spray on net photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	19.70c	18.30b
	0.1	20.80b	18.60b
	0.5	23.40a	21.10a
	1.0	18.90d	17.10c
	NaCl (50mM)		
	SA (mM)		
	0.0	17.10f	14.10e
	0.1	17.70e	14.20e
	0.5	19.40c	15.50d
	1.0	14.70g	11.20f
	LSD at $P<0.05$	0.47	0.50
60	NaCl (0mM)		
	SA (mM)		
	0.0	21.80c	20.40c
	0.1	23.30b	21.00b
	0.5	26.10a	23.60a
	1.0	20.90d	19.00d
	NaCl (50mM)		
	SA (mM)		
	0.0	19.20f	16.60f
	0.1	20.10e	16.90f
	0.5	22.00c	18.40e
	1.0	16.30g	13.10g
	LSD at $P<0.05$	0.44	0.49
90	NaCl (0mM)		
	SA (mM)		
	0.0	20.30d	19.00c
	0.1	21.90b	19.70b
	0.5	24.50a	22.20a
	1.0	19.40e	17.70d
	NaCl (50mM)		
	SA (mM)		
	0.0	18.00g	15.90e
	0.1	19.00f	16.30e
	0.5	20.80c	17.80d
	1.0	15.10h	12.40f
	LSD at $P<0.05$	0.45	0.43

Different letters within a column indicate significant difference at $P<0.05$.

Table 56: Effect of salicylic acid (SA) spray on stomatal conductance (m mol m^{-2} s^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	415.00b	400.00b
	0.1	440.00b	408.00b
	0.5	494.00a	460.00a
	1.0	398.00d	374.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	364.00e	313.00e
	0.1	379.00e	317.00e
	0.5	414.00c	345.00d
	1.0	312.00f	250.00f
	LSD at $P<0.05$	9.62	10.02
60	NaCl (0mM)		
	SA (mM)		
	0.0	427.00c	406.00b
	0.1	457.00b	419.00b
	0.5	513.00a	471.00a
	1.0	410.00d	380.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	381.00e	335.00e
	0.1	401.00e	342.00e
	0.5	439.00b	373.00d
	1.0	323.00f	264.00f
	LSD at $P<0.05$	10.04	10.04
90	NaCl (0mM)		
	SA (mM)		
	0.0	410.00c	396.00b
	0.1	443.00b	412.00b
	0.5	497.00a	464.00a
	1.0	394.00d	370.00d
	NaCl (50mM)		
	SA (mM)		
	0.0	368.00e	336.00e
	0.1	391.00e	347.00e
	0.5	428.00b	377.00c
	1.0	308.00f	261.00f
	LSD at $P<0.05$	10.29	9.49

Different letters within a column indicate significant difference at $P<0.05$.

Table 57: Effect of salicylic acid (SA) spray on intercellular CO_2 concentration (μ mol mol^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	283.98b	281.07b
	0.1	301.59b	287.25b
	0.5	338.50a	323.79a
	1.0	272.62d	262.80c
	NaCl (50mM)		
	SA (mM)		
	0.0	243.37e	216.14e
	0.1	253.35e	218.52e
	0.5	277.69c	237.97d
	1.0	209.05f	172.70f
	LSD at $P<0.05$	6.40	7.17
60	NaCl (0mM)		
	SA (mM)		
	0.0	287.98c	285.26b
	0.1	288.19b	294.67b
	0.5	346.15a	331.47a
	1.0	276.46d	266.72c
	NaCl (50mM)		
	SA (mM)		
	0.0	250.54e	229.06e
	0.1	263.32e	233.87e
	0.5	288.37b	254.49d
	1.0	212.71f	180.73f
	LSD at $P<0.05$	6.59	7.22
90	NaCl (0mM)		
	SA (mM)		
	0.0	286.36c	284.22b
	0.1	309.84b	296.16b
	0.5	347.07a	333.11a
	1.0	274.91d	265.75c
	NaCl (50mM)		
	SA (mM)		
	0.0	250.85e	234.77e
	0.1	266.15e	242.05e
	0.5	291.24b	263.18d
	1.0	210.46f	182.89f
	LSD at $P<0.05$	6.90	7.13

Different letters within a column indicate significant difference at $P<0.05$.

Table 58: Effect of salicylic acid (SA) spray on chlorophyll content (mg g^{-1} fresh mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	1.74c	1.70b
	0.1	1.84b	1.73b
	0.5	2.07a	1.95a
	1.0	1.67d	1.58c
	NaCl (50mM)		
	SA (mM)		
	0.0	1.51e	1.31e
	0.1	1.57e	1.32e
	0.5	1.72cd	1.44d
	1.0	1.29f	1.04f
	LSD at $P<0.05$	0.06	0.03
60	NaCl (0mM)		
	SA (mM)		
	0.0	2.00cd	1.92b
	0.1	2.14b	1.97b
	0.5	2.40a	2.22a
	1.0	1.92de	1.97b
	NaCl (50mM)		
	SA (mM)		
	0.0	1.76f	1.56d
	0.1	1.84ef	1.59d
	0.5	2.02c	1.73c
	1.0	1.49g	1.23e
	LSD at $P<0.05$	0.09	0.06
90	NaCl (0mM)		
	SA (mM)		
	0.0	2.19cd	2.07b
	0.1	2.36b	2.15b
	0.5	2.64a	2.42a
	1.0	2.10de	1.93c
	NaCl (50mM)		
	SA (mM)		
	0.0	1.94f	1.73d
	0.1	2.05e	1.78d
	0.5	2.25c	1.93c
	1.0	1.62g	1.34e
	LSD at $P<0.05$	0.09	0.08

Different letters within a column indicate significant difference at $P<0.05$.

Table 59: Effect of salicylic acid (SA) spray on carotenoid content (mg g^{-1} fresh mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	0.93c	0.91b
	0.1	0.98b	0.93b
	0.5	1.11a	1.05a
	1.0	0.89d	0.85c
	NaCl (50mM)		
	SA (mM)		
	0.0	0.80f	0.70e
	0.1	0.83e	0.71e
	0.5	0.91cd	0.77d
	1.0	0.69g	0.56f
	LSD at $P<0.05$	0.02	0.02
60	NaCl (0mM)		
	SA (mM)		
	0.0	1.27c	1.21c
	0.1	1.36b	1.25b
	0.5	1.52a	1.40a
	1.0	1.22d	1.13d
	NaCl (50mM)		
	SA (mM)		
	0.0	1.12f	0.99e
	0.1	1.18e	1.01e
	0.5	1.29c	1.10d
	1.0	0.95g	0.78f
	LSD at $P<0.05$	0.03	0.03
90	NaCl (0mM)		
	SA (mM)		
	0.0	2.01d	1.90c
	0.1	2.17b	1.98b
	0.5	2.43a	2.22a
	1.0	1.93e	1.78d
	NaCl (50mM)		
	SA (mM)		
	0.0	1.78f	1.59e
	0.1	1.89e	1.64e
	0.5	2.06c	1.78d
	1.0	1.49g	1.24f
	LSD at $P<0.05$	0.04	0.05

Different letters within a column indicate significant difference at $P<0.05$.

increased significantly with salinity at all sampling times. Accumulation of sodium and chloride was higher in PBM16 than Alankar under saline and non-saline conditions. However, in both the cultivars, application of SA had significant effect in restricting the high accumulation of sodium and chloride compared to control. An application of 0.5mM SA proved most effective in reducing the concentrations of sodium and chloride.

The application of SA decreased sodium and chloride concentrations under non-saline condition. The decreases in sodium and chloride concentrations were 30.43 and 31.25% with 0.5mM SA in Alankar whereas the decreases in PBM16 were 25.76 and 25.61% at 30DAS; 35.56 and 35.63% in Alankar and 18.33 and 18.26% in PBM16 at 60DAS, and 37.25 and 36.86% in Alankar and 17.04 and 17.16% in PBM16 at 90DAS in comparison to the control (0mM NaCl plus 0mM SA).

The treatment of 50mM NaCl increased sodium and chloride concentrations in Alankar and PBM16. The increases due to 50mM NaCl in these elements in Alankar were 13.04 and 12.50% at 30DAS; 3.33 and 11.49% at 60DAS, and 4.90 and 7.89% at 90DAS. A higher increase in the above elements was observed in PBM16, which was 15.15 and 14.63% at 30DAS; 5.83 and 14.78% at 60DAS, and 8.89 and 11.19% at 90DAS.

The effects of 50mM NaCl were reversed with the application of 0.5mM SA. The application of 0.5mM SA on plants fed with 50mM NaCl decreased the concentrations of sodium and chloride in comparison to the control. The decreases in sodium and chloride concentrations were 26.09 and 26.56% in Alankar and 19.70 and 20.73% in PBM16 at 30DAS; 3.33 and 28.74% in Alankar and 19.17 and 12.17% in PBM16 at 60DAS, and 3.33 and 31.58% in Alankar and 16.30 and 14.18% in PBM16 at 90DAS.

Application of SA had significant effect on nitrogen, phosphorus, potassium and calcium concentrations, but statistically non-significant on calcium concentration at 30DAS (Tables 62-65). Nitrogen, phosphorus,

potassium and calcium concentrations decreased significantly with salinity. These nutrient concentrations were significantly greater in Alankar than PBM16. Further, 0.5mMSA application also enhanced the nutrient concentrations under both saline and non-saline conditions.

The application of 0.5mM SA showed significant increase in nitrogen, phosphorus, potassium and calcium concentrations. The increases in nitrogen, phosphorus, potassium and calcium concentrations with 0.5mM SA application in Alankar were 32.76, 32.68, 32.58 and 33.00% at 30DAS; 31.08, 30.98, 30.81 and 30.83% at 60DAS, and 30.15, 30.23, 30.05 and 30.00% at 90DAS. The cultivar PBM16 showed lesser increase with 0.5mM SA, which was 29.41, 29.21, 29.17 and 28.57% at 30DAS; 28.36, 28.31, 28.17 and 28.46% at 60DAS, and 28.10, 27.87, 28.10 and 27.70% at 90DAS compared to the control.

Nitrogen, phosphorus, potassium and calcium concentrations increased to a significant extent over control with SA application under saline and non-saline conditions. In plants treated with 50mM NaCl, the concentrations of nitrogen, phosphorus, potassium and calcium were decreased to 6.03, 46.46, 4.49 and 13.00% at 30DAS; 6.76, 19.87, 5.95 and 11.28% at 60DAS, and 8.09, 12.65, 6.56 and 8.00% at 90DAS in Alankar and 7.84, 64.85, 5.56 and 18.68% at 30DAS; 7.46, 23.35, 7.75 and 16.26% at 60DAS, and 9.09, 13.31, 7.19 and 10.81% at 90DAS in PBM16 compared to control.

The combined treatment of 50mM NaCl and 0.5mM SA increased nitrogen concentration by 23.28, 10.14 and 0.74% in Alankar and 13.73, 5.22 and 0.00% (became equal to control) in PBM16 at 30, 60 and 90DAS, respectively compared to respective control. In Alankar and PBM16, the decrease in phosphorus concentration due to the combined treatment 0.5mM SA plus 50mM NaCl was less compared to the treatment 50mM NaCl alone. The decreases due to the treatment 0.5mM SA plus 50mM NaCl in the two cultivars were limited to 29.92 and 56.93% at 30DAS; 5.56 and 12.50% at

Table 60: Effect of salicylic acid (SA) spray on sodium concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	4.60b	6.60c
	0.1	3.60e	5.40f
	0.5	3.20g	4.90g
	1.0	4.20c	6.30d
	NaCl (50mM)		
	SA (mM)		
	0.0	5.20a	7.60a
	0.1	3.90d	5.80e
	0.5	3.40f	5.30f
	1.0	4.50b	6.90b
	LSD at $P<0.05$	0.10	0.17
60	NaCl (0mM)		
	SA (mM)		
	0.0	9.00	12.00b
	0.1	6.50	10.00d
	0.5	5.80	9.80fg
	1.0	7.70	11.80c
	NaCl (50mM)		
	SA (mM)		
	0.0	9.30	12.70a
	0.1	6.60	9.90de
	0.5	6.00	9.70g
	1.0	8.10	12.10b
	LSD at $P<0.05$	NS	0.18
90	NaCl (0mM)		
	SA (mM)		
	0.0	10.20b	13.50c
	0.1	7.10f	11.50e
	0.5	6.40h	11.20f
	1.0	8.50d	13.40c
	NaCl (50mM)		
	SA (mM)		
	0.0	10.70a	14.70a
	0.1	7.40e	11.80d
	0.5	6.80g	11.30ef
	1.0	9.40c	14.40b
	LSD at $P<0.05$	0.16	0.25

Different letters within a column indicate significant difference at $P<0.05$.

Table 61: Effect of salicylic acid (SA) spray on chloride concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	6.40b	8.20c
	0.1	5.10e	6.70f
	0.5	4.40g	6.10h
	1.0	5.90c	7.90d
	NaCl (50mM)		
	SA (mM)		
	0.0	7.20a	9.40a
	0.1	5.30d	7.20e
	0.5	4.70f	6.50g
	1.0	6.30b	8.60b
	LSD at $P<0.05$	0.14	0.17
60	NaCl (0mM)		
	SA (mM)		
	0.0	8.70b	11.50c
	0.1	6.30f	9.70g
	0.5	5.60g	9.40h
	1.0	7.40d	11.30d
	NaCl (50mM)		
	SA (mM)		
	0.0	9.70a	13.20a
	0.1	6.90e	10.30e
	0.5	6.20f	10.10f
	1.0	8.50c	12.60b
	LSD at $P<0.05$	0.19	0.17
90	NaCl (0mM)		
	SA (mM)		
	0.0	11.40b	13.40c
	0.1	8.00f	11.40e
	0.5	7.20h	11.10f
	1.0	9.50d	13.30c
	NaCl (50mM)		
	SA (mM)		
	0.0	12.30a	14.90a
	0.1	8.50e	11.90d
	0.5	7.80g	11.50e
	1.0	10.70c	14.60b
	LSD at $P<0.05$	0.18	0.25

Different letters within a column indicate significant difference at $P<0.05$.

Table 62: Effect of salicylic acid (SA) spray on nitrogen concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	11.60e	10.20d
	0.1	13.80c	11.20c
	0.5	15.40a	13.20a
	1.0	12.00d	9.90e
	NaCl (50mM)		
	SA (mM)		
	0.0	10.90f	9.40f
	0.1	12.10d	10.30d
	0.5	14.30b	11.60b
	1.0	10.60g	9.00g
	LSD at $P<0.05$	0.25	0.21
60	NaCl (0mM)		
	SA (mM)		
	0.0	14.80d	13.40c
	0.1	16.10b	14.20b
	0.5	19.40a	17.20a
	1.0	15.20c	13.20cd
	NaCl (50mM)		
	SA (mM)		
	0.0	13.80e	12.40e
	0.1	14.90cd	13.10d
	0.5	16.30b	14.10b
	1.0	13.50e	12.10f
	LSD at $P<0.05$	0.34	0.25
90	NaCl (0mM)		
	SA (mM)		
	0.0	13.60c	12.10c
	0.1	14.70b	12.50b
	0.5	17.70a	15.50a
	1.0	13.90c	12.00c
	NaCl (50mM)		
	SA (mM)		
	0.0	12.50d	11.00e
	0.1	13.50c	11.30d
	0.5	13.70cd	12.10c
	1.0	12.20e	10.70f
	LSD at $P<0.05$	0.29	0.23

Different letters within a column indicate significant difference at $P<0.05$.

Table 63: Effect of salicylic acid (SA) spray on phosphorus concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	25.40d	20.20bc
	0.1	30.20b	22.10b
	0.5	33.70a	26.10a
	1.0	26.40c	19.60c
	NaCl (50mM)		
	SA (mM)		
	0.0	13.60g	7.10ef
	0.1	15.10f	7.80e
	0.5	17.80e	8.70d
	1.0	13.20g	6.80f
	LSD at $P<0.05$	0.86	0.80
60	NaCl (0mM)		
	SA (mM)		
	0.0	59.40d	54.40c
	0.1	64.70b	57.50b
	0.5	77.80a	69.80a
	1.0	61.00c	53.50c
	NaCl (50mM)		
	SA (mM)		
	0.0	47.60g	41.70f
	0.1	51.40f	44.00e
	0.5	56.10e	47.60d
	1.0	46.40g	40.60f
	LSD at $P<0.05$	1.36	1.22
90	NaCl (0mM)		
	SA (mM)		
	0.0	93.30c	87.90c
	0.1	100.80b	90.50b
	0.5	121.50a	112.40a
	1.0	95.50bc	87.30c
	NaCl (50mM)		
	SA (mM)		
	0.0	81.50ef	76.20f
	0.1	87.80de	78.50e
	0.5	89.60cd	83.80d
	1.0	79.80f	74.40g
	LSD at $P<0.05$	6.78	1.79

Different letters within a column indicate significant difference at $P<0.05$.

Table 64: Effect of salicylic acid (SA) spray on potassium concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	8.90f	7.20e
	0.1	10.60c	7.90c
	0.5	11.80a	9.30a
	1.0	9.20e	7.00f
	NaCl (50mM)		
	SA (mM)		
	0.0	8.50g	6.80g
	0.1	9.50d	7.40d
	0.5	11.10b	8.40b
	1.0	8.20h	6.50h
	LSD at $P<0.05$	0.23	0.19
60	NaCl (0mM)		
	SA (mM)		
	0.0	18.50d	14.20c
	0.1	20.20b	15.00b
	0.5	24.20a	18.20a
	1.0	19.00c	13.90d
	NaCl (50mM)		
	SA (mM)		
	0.0	17.40e	13.10e
	0.1	18.80cd	13.80d
	0.5	20.50b	14.90b
	1.0	17.00f	12.70f
	LSD at $P<0.05$	0.39	0.27
90	NaCl (0mM)		
	SA (mM)		
	0.0	18.30e	15.30c
	0.1	19.80b	15.70b
	0.5	23.80a	19.60a
	1.0	18.70cd	15.20d
	NaCl (50mM)		
	SA (mM)		
	0.0	17.10f	14.20f
	0.1	18.40de	14.60e
	0.5	18.80c	15.60bc
	1.0	16.70g	13.90f
	LSD at $P<0.05$	0.36	0.33

Different letters within a column indicate significant difference at $P<0.05$.

Table 65: Effect of salicylic acid (SA) spray on calcium concentration (mg g^{-1} dry mass) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30, 60 and 90 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
30	NaCl (0mM)		
	SA (mM)		
	0.0	10.00	9.10
	0.1	11.90	10.00
	0.5	13.30	11.70
	1.0	10.40	8.80
	NaCl (50mM)		
	SA (mM)		
	0.0	8.70	7.40
	0.1	9.70	8.10
	0.5	11.40	9.10
	1.0	8.40	7.10
	LSD at $P<0.05$	NS	NS
60	NaCl (0mM)		
	SA (mM)		
	0.0	13.30e	12.30c
	0.1	14.50b	13.00b
	0.5	17.40a	15.80a
	1.0	13.60d	12.10c
	NaCl (50mM)		
	SA (mM)		
	0.0	11.80g	10.30f
	0.1	12.70f	10.90e
	0.5	13.90c	11.70d
	1.0	11.50h	10.00g
	LSD at $P<0.05$	0.27	0.24
90	NaCl (0mM)		
	SA (mM)		
	0.0	15.00de	14.80c
	0.1	16.20b	15.20b
	0.5	19.50a	18.90a
	1.0	15.40c	14.70cd
	NaCl (50mM)		
	SA (mM)		
	0.0	13.80f	13.20f
	0.1	14.90e	13.60e
	0.5	15.20cd	14.50d
	1.0	13.50f	12.90g
	LSD at $P<0.05$	0.34	0.28

Different letters within a column indicate significant difference at $P<0.05$.

60DAS, and 3.97 and 4.66%, respectively at 90DAS. Moreover, potassium content with the combined application of 0.5mM SA and 50mM NaCl increased in comparison to control at all sampling times. In Alankar and PBM16, the increase in the potassium concentration was 24.72 and 16.67% at 30DAS; 10.81 and 4.93% at 60DAS, and 2.73 and 1.96% at 90DAS. In Alankar, the application of 0.5mM SA reversed the adverse effect of 50mM NaCl on calcium concentration and increased it by 14.00, 4.51 and 1.33% at 30, 60 and 90DAS, respectively in comparison to control. However, in PBM16 also the application of 0.5mM SA reversed the adverse effect of 50mM NaCl on calcium concentration and the concentrations were lower than the control.

4.3.2.4 Activities of antioxidative enzymes

Activities of antioxidative enzymes increased with the salinity treatment, and was greater in tolerant (Alankar) than in non-tolerant (PBM16) cultivar at 30DAS, the time of its measurement. The activities of antioxidative enzymes were also increased with the application of SA under both saline and non-saline conditions (Tables 66-67).

Activities of antioxidative enzymes of both the cultivars increased significantly with SA treatment. The cultivars, Alankar and PBM16 showed an enhancement of 34.80 and 31.40% in catalase activity, 34.70 and 31.50% in superoxide dismutase activity, 31.82 and 33.33% in glutathione reductase activity and 34.67 and 30.91% in ascorbate peroxidase activity with the application of 0.5 mM SA (Tables 66-67).

Activities of antioxidative enzymes of both the cultivars increased significantly with 50mM NaCl. At 50mM NaCl, per cent increases in the catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase activities in Alankar were 10.74, 6.67, 4.55 and 8.00%, respectively. In PBM16, catalase activity was increased by 16.07%, superoxide dismutase activity by 10.00%, glutathione reductase activity by 5.56% and ascorbate peroxidase activity by 6.36% at 30DAS due to 50mM NaCl.

Table 66: Effect of salicylic acid (SA) spray on catalase activity (U g^{-1} FM min^{-1}) and superoxide dismutase activity (U mg^{-1} protein) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
		Catalase	
30	NaCl (0mM)		
	SA (mM)		
	0.0	121.00f	112.00g
	0.1	152.34c	134.96d
	0.5	163.11b	147.17c
	1.0	106.24g	102.26h
	NaCl (50mM)		
	SA (mM)		
	0.0	134.00d	130.00e
	0.1	165.76a	154.31b
	0.5	168.30a	163.54a
	1.0	127.57e	123.89f
	LSD at P<0.05	3.12	2.88
		Superoxide dismutase	
30	NaCl (0mM)		
	SA (mM)		
	0.0	30.00f	20.00f
	0.1	37.62d	24.12c
	0.5	40.41b	26.30b
	1.0	26.22g	18.24g
	NaCl (50mM)		
	SA (mM)		
	0.0	32.00e	22.00d
	0.1	39.52c	26.05b
	0.5	41.79a	27.65a
	1.0	29.57f	20.99e
	LSD at $P<0.05$	0.78	0.49

Different letters within a column indicate significant difference at $P<0.05$.

Table 67: Effect of salicylic acid (SA) spray on glutathione reductase activity (U mg^{-1} protein) and ascorbate peroxidase activity (U mg^{-1} protein) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at 30 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
		Glutathione reductase	
30	NaCl (0mM)		
	SA (mM)		
	0.0	0.215f	0.180d
	0.1	0.270d	0.217b
	0.5	0.290b	0.237a
	1.0	0.188g	0.165e
	NaCl (50mM)		
	SA (mM)		
	0.0	0.227e	0.185c
	0.1	0.280c	0.218b
	0.5	0.297a	0.233a
	1.0	0.210f	0.176d
	LSD at P<0.05	0.006	0.004
		Ascorbate peroxidase	
30	NaCl (0mM)		
	SA (mM)		
	0.0	1.50e	1.10e
	0.1	1.88c	1.33c
	0.5	2.02b	1.44b
	1.0	1.32f	1.01f
	NaCl (50mM)		
	SA (mM)		
	0.0	1.62d	1.17d
	0.1	2.00b	1.38b
	0.5	2.12a	1.47a
	1.0	1.50e	1.12e
	LSD at $P<0.05$	0.04	0.03

Different letters within a column indicate significant difference at $P<0.05$.

The activities of antioxidative enzymes were significantly further increased when 50mM NaCl was supplemented with 0.5mM SA. In Alankar, the increase in catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase activities due to the combined treatment 0.5mM SA plus 50mM NaCl was more compared with the treatment of 50mM NaCl alone, and the increases were 39.09, 39.30, 36.36 and 41.33%, respectively. However, in PBM16, the increment in catalase activity was 46.02%, superoxide dismutase activity was 38.25%, glutathione reductase activity was 27.78% and ascorbate peroxidase activity was 33.64% due to the treatment 50mM NaCl plus 0.5mM SA.

4.3.2.5 Yield characteristics

Salt stress led to a significant reduction in yield characteristics. However, SA application increased the yield characteristics and alleviated the salt stress effects when applied as a combined treatment of NaCl and SA. The application of 0.5mM SA proved most effective (Tables 68-69).

Yield characteristics were increased by SA application and were greater in non-salinized (0mM NaCl) than in salinized conditions (50mM NaCl). At 0mM NaCl, the yield characteristics in both the cultivars increased significantly with SA application. With 0.5mM SA application, pod length of Alankar and PBM16 was increased by 19.61 and 15.38%, pod number by 21.00 and 17.00%, seed number by 20.95 and 16.98% and seed yield by 20.99 and 17.01% in comparison to control.

Pod length, pod number, seed number and seed yield of Alankar were decreased by 13.73, 13.09, 13.47 and 13.51% in response to 50mM NaCl treatment in comparison to control. The above characteristics of PBM16 were decreased by 28.21, 29.40, 29.32 and 29.30% respectively, in response to 50mM NaCl.

Application of 0.5mM SA alleviated the decrease of yield characteristics under saline condition (50mM NaCl). In Alankar, the 0.5mM SA treatment

Table 68: Effect of salicylic acid (SA) spray on pod length (cm) and pod number plant^{-1} of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at harvest, i.e., 120 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
		Pod length	
120	NaCl (0mM)		
	SA (mM)		
	0.0	5.10c	3.90b
	0.1	5.50b	4.00b
	0.5	6.10a	4.50a
	1.0	5.20c	3.80b
	NaCl (50mM)		
	SA (mM)		
	0.0	4.40e	2.80d
	0.1	4.60d	2.80d
	0.5	5.10c	3.10c
	1.0	3.60f	2.10e
	LSD at $P<0.05$	0.17	0.21
		Pod number	
120	NaCl (0mM)		
	SA (mM)		
	0.0	105.18c	89.68c
	0.1	113.59b	93.27b
	0.5	127.27a	104.93a
	1.0	107.28c	89.23c
	NaCl (50mM)		
	SA (mM)		
	0.0	91.41e	63.31e
	0.1	96.89d	65.21e
	0.5	106.04c	72.81d
	1.0	76.78f	49.38f
	LSD at $P<0.05$	3.16	3.45

Different letters within a column indicate significant difference at $P<0.05$.

Table 69: Effect of salicylic acid (SA) spray on seed number pod^{-1} and seed yield (g plant^{-1}) of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) grown under salinity stress at harvest, i.e., 120 days after sowing (DAS).

DAS	Treatments	Alankar	PBM16
		Seed number	
120	NaCl (0mM)		
	SA (mM)		
	0.0	12.03c	10.13c
	0.1	12.99b	10.53b
	0.5	14.55a	11.85a
	1.0	12.27c	10.07c
	NaCl (50mM)		
	SA (mM)		
	0.0	10.41e	7.16e
	0.1	11.03d	7.37e
	0.5	12.07c	8.01d
	1.0	8.74f	5.58f
	LSD at $P<0.05$	0.33	0.36
		Seed yield	
120	NaCl (0mM)		
	SA (mM)		
	0.0	6.29c	4.88bc
	0.1	6.79b	5.07ab
	0.5	7.61a	5.71a
	1.0	6.41c	4.85c
	NaCl (50mM)		
	SA (mM)		
	0.0	5.44e	3.45e
	0.1	5.76d	3.55e
	0.5	6.31c	3.86d
	1.0	4.57f	2.69f
	LSD at $P<0.05$	0.19	0.19

Different letters within a column indicate significant difference at $P<0.05$.

reversed the effects of 50mM NaCl and the reduction in the characteristics was restricted to about near control. Pod length was equivalent to the control plants whereas pod number, seed number and seed yield showed a marginal increase of 0.82, 0.33 and 0.32%, respectively.

The decreases in pod length, pod number, seed number and seed yield due to 50mM NaCl were observed, however, lesser decreases were found when 50mM NaCl treatment was supplemented with 0.5 mM SA.

Contrary to the results obtained in Alankar (where 0.5mM SA plus 50mM NaCl increased the characteristics in comparison to 50mM NaCl alone), PBM16 exhibited decreases which were limited to 20.51, 18.81, 20.93 and 20.90% in pod length, pod number, seed number and seed yield, respectively due to 0.5mM SA plus 50mM NaCl compared to 50mM NaCl.

4.4 Experimental Summary

4.4.1 Experiment 1

- The effect of 100mM NaCl decreased the growth and photosynthetic characteristics maximally and was more conspicuous on all the cultivars of mungbean at 20 and 40DAS sampling times.
- The effect of 100mM NaCl on yield characteristics was detrimental and the plants could not survive at maturity.
- Plants treated with 50mM NaCl exhibited a significant decrease over control on growth, photosynthetic and yield characteristics.
- Among cultivars, Tram exhibited greatest decrease in the growth, photosynthetic and yield characteristics due to the salinity treatments, whereas Pusa Vishal showed lowest decrease.
- The order of suitability of the cultivars to salinity stress in terms of growth, photosynthetic and yield characteristics was Pusa Vishal > PDM54 > T44 > Tram.

4.4.2 Experiment 2

- Maximum reduction in the growth and photosynthetic characteristics was noted with 100mM NaCl at all the sampling times in all the cultivars of mustard. However, treatment of 100mM NaCl proved deleterious and plants did not survive up to maturity.
- Growth, photosynthetic and yield reductions were significantly greater in Sakha and PBM16 than the Alankar and Pusa Bold with NaCl concentrations.
- The order of tolerance of the cultivars to salinity stress was Alankar > Pusa Bold > Sakha > PBM16.

4.4.3 Experiment 3

- Application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations, activities of antioxidative enzymes and yield characteristics of Pusa Vishal (tolerant mungbean type) and Tram (non-tolerant mungbean type) cultivars grown under non-saline (control) condition.
- Non-salinized plants treated with 0.5mM SA maintained a higher growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics than salinized plants at both the stages, indicating adverse effects of NaCl salinity in tolerant (Pusa Vishal) as well as non-tolerant (Tram) cultivars.
- Application of 0.5mM SA decreased the concentrations of sodium and chloride in both tolerant (Pusa Vishal) and non-tolerant (Tram) cultivars, under normal and saline conditions at both the sampling times.
- Application of 0.5mM SA increased the activities of antioxidative enzymes of plants grown under non-saline or salinized conditions.

- Growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics decreased significantly with 50mM NaCl in both the cultivars but more adverse effects of salinity were found on Tram. However, the concentrations of sodium and chloride and the activities of antioxidative enzymes increased with 50mM NaCl in both the cultivars.
- The treatment of 0.5mM SA was found most effective in alleviating salinity stress on growth, photosynthetic, biochemical and yield characteristics.

4.4.4 Experiment 4

- The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mustard. In both the cultivars i.e. Alankar and PBM16 the increases were greater under non-saline (control) condition than under saline condition at all sampling times.
- The positive effect of 0.5mM SA application was found as it decreased sodium and chloride concentrations under both saline and non-saline conditions.
- The activities of antioxidative enzymes of both the cultivars increased significantly with SA under both saline and non-saline conditions.
- Salt stress led to a significant reduction in growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of both the cultivars. The cultivar PBM16 exhibited a higher reduction than Alankar.
- The treatment of 50mM NaCl increased sodium and chloride concentrations in Alankar and PBM16 at all the sampling times, and the accumulation was higher in PBM16 than Alankar.

- Exposure of plants to 50mM NaCl increased the activities of antioxidative enzymes in both the cultivars but to a higher degree in Alankar than PBM16.
- Application of 0.5mM SA helped to reduce the adverse effects of salinity. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl.

DISCUSSION

Contents

DISCUSSION

DISCUSSION

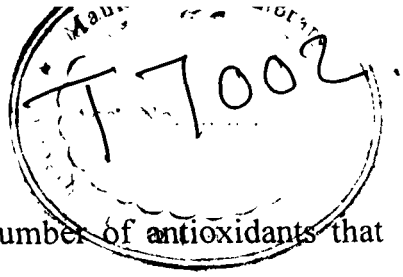
5.1 Introduction

An environmental factor that limits biomass production and crop productivity is referred to as stress or disturbance (Grime, 1979). Salinity in soil or water is one of the major stresses in arid and semi-arid regions limiting crop productivity severely (Shannon, 1998). The deleterious effects of salinity on plants are associated with (1) low osmotic potential of soil solution, (2) nutritional imbalance, or (3) specific ion effect (salt stress) (Ashraf, 1994; Marschner, 1995). All of these as individual or combined factor cause adverse pleiotrophic effects on plant growth and development at physiological and biochemical (Levitt, 1980; Gorham *et al.*, 1985; Munns, 2002) or at molecular level (Winicov, 1998; Mansour, 2000; Tester and Davenport, 2003). Soil salinity is a measure of the total amount of soluble salt, and as the level of salinity increases, plants extract water less easily from the growing medium aggravating water stress conditions. Salinity can cause nutrient imbalances, resulting in the accumulation of elements toxic to plants, and reduce water infiltration if the level of one salt element, sodium becomes high.

For various kinds of environmental stresses, such as salinity, drought, water logging, temperature extremes, high light intensity, herbicide treatment or mineral nutrient deficiency the production of active oxygen species (AOS) is associated with the deleterious effects on plant functions (Wise and Naylor, 1987; Monk and Davies, 1989; Catmak and Marschner, 1992; Gossett *et al.*, 1994; Mittova *et al.*, 2000; Mittova *et al.*, 2002). Salinity stress in plants also impairs photosynthetic process and exerts deleterious effects on plant growth and development via the production of AOS because of the impairment in the carbon dioxide reduction and production of NADPH in electron transport chain. In chloroplasts, AOS can be generated by direct transfer of excitation energy from chlorophyll to produce singlet oxygen, or by univalent oxygen

reduction photosystem I in the Mehler reaction (Asada, 1999). AOS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids. Under stress conditions, plants activate mechanisms that help in reduction of the effects of AOS.

Plants operate several mechanisms, like production of osmolytes, homeostasis of ions, activation of antioxidative system (enzymatic and non-enzymatic) and the interaction of plant growth regulators with stress to counteract the adverse effects. Among osmolytes, proline is considered to regulate the accumulation of useable N (Wyn Jones, 1981; Ashraf, 1994), contributes the membrane stability (Rudolph *et al.*, 1986; Lone *et al.*, 1987; Hanson and Burnet, 1994; Gadallah, 1999) and mitigates the effects of NaCl on cell membrane disruption (Mansour, 1998). Maggio *et al.* (2002) are of the view that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process. Another important compound is glycine-betaine, mainly localized in chloroplasts and involved in the adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986; Genard *et al.*, 1991). Murata *et al.* (1992) reported that glycine-betaine protects the PSII complex by stabilizing the association of the extrinsic PSII complex proteins under salt stress. The capability of plants for adjustment of salts in plant cell organelle or salt avoidance has been worked out for tolerance of plants. Ion uptake and compartmentalization are crucial for tolerance against saline conditions (Adams *et al.*, 1992b) because the stress disturbs ion homeostasis. Plants growing under high saline conditions restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Reddy *et al.*, 1992; Iyengar and Reddy, 1996; Zhu, 2003) or limit sodium uptake or partition sodium in older tissues that serve as storage compartments that are eventually sacrificed (Cheeseman, 1988).



In varying degrees, plants also possess a number of antioxidants that protect plants against the potentially cytotoxic ASO. The metalloenzyme superoxide dismutase converts $O_2^{\bullet-}$ to H_2O_2. Catalase and a variety of peroxidases (Chang *et al.*, 1984) catalyze the breakdown of H_2O_2. Although catalase is apparently absent in the chloroplast, H_2O_2 can be detoxified in a reaction catalyzed by an ascorbate-specific peroxidase often present in high levels of this organelle (Chen and Asada, 1989) through the ascorbate-glutathione cycle (Halliwell and Gutteridge, 1986; Asada, 1992). Ascorbate can also be oxidized by direct reaction with $O_2^{\bullet-}$ or by serving as a reductant of the α-chromoxyl radical of oxidized α-tocopherol (Foyer *et al.*, 1991). The thylakoid membranes are rich in α-tocopherol which disrupts lipid peroxidation reactions not only by reacting with $O_2^{\bullet-}$ but also by scavenging hydroxyl, peroxyl, and alkoxyl radicals (Halliwell, 1987). Plants containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the activated oxygen species (Wise and Naylor, 1987; Spychalla and Desborough, 1990; Shalata and Tal, 1998; Garratt *et al.*, 2002).

The interaction of salt stress with phytohormones has been found. The change in phytohormone level has been correlated with salt stress tolerance. High salt concentration triggers an increase in levels of plant hormones such as abscisic acid (ABA) and cytokinins (Thomas *et al.*, 1992; Aldesuquy, 1998; Vaidyanathan *et al.*, 1999). ABA is responsible for the alteration of salt stress-induced genes (de Bruxelles *et al.*, 1996). ABA promotes stomatal closure by rapidly altering ion fluxes in guard cells under stress conditions. Experimental evidence shows that the increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term (Chen *et al.*, 2001).

Salicylic acid, (a plant hormone considered as naturally occurring hormone) is involved in plant defense under various kind of stress. SA has received particular attention because its accumulation is essential for expression of multiple modes of plant disease resistance (Shirasu *et al.*, 1997). SA drew the attention of researchers due to its ability to induce systemic acquired resistance in plants to different pathogens, which is manifested in the appearance of pathogenesis related proteins and SA serves as a signal in the induction of expression of these genes (Metraux, 2001). Several studies supported a major role of SA in modulating the plant response to abiotic stresses, such as ultraviolet light, drought, salt, chilling, heat, etc. (Hamada, 1998; Janda *et al.*, 1999; Mishra and Choudhuri, 1999; Dat *et al.*, 2000; Al-Hakimi and Hamada, 2001). SA is of considerable importance in the regulation of plant growth and metabolism (Vendrig and Buffel, 1961; Wain and Taylor, 1965). Its role as a plant morphogenetic regulator, as a flower inducing factor, is well established (Cleland and Ajami, 1974; Asthana and Srivastava, 1978; Watanabe and Takimoto, 1979). SA accumulates during exposure to ozone and UV light (Yalpani *et al.*, 1994; Sharma *et al.*, 1996). Pretreatment of leaves with SA can protect them from paraquat-induced oxidative stress (Strobel and Kuc, 1995).

Keeping in view the importance of SA in plant development under normal and stress conditions, the present work was taken up with the following objectives.

1. To compare the effects of salinity stress on four cultivars of mungbean and mustard, and select tolerant and non-tolerant cultivars on the basis of their growth, photosynthetic and yield characteristics.
2. To study the effects of salicylic acid in alleviating salinity stress and physiological and biochemical changes associated with the application of salicylic acid in tolerant and non-tolerant cultivars of mungbean and mustard.

5.2 Comparison of Cultivars Performance under Salinity Stress

Experiments 1 and 2 were conducted to compare the effects of salinity stress on four cultivars of mungbean and mustard, respectively. On the basis of growth, photosynthetic and yield performance, tolerant and non-tolerant cultivars of mungbean and mustard were selected. Plants subjected to 50 and 100mM NaCl reduced growth and photosynthetic characteristics in comparison to control. Maximum reduction in the characteristics was found in plants grown with 100mM NaCl in both the crops (Tables 4-12,14-22). In mungbean and mustard, plants treated with 50mM NaCl exhibited a significant decrease over control on yield characteristics (Tables 13,23).

The order of performance of cultivars was Pusa Vishal > PDM54 > T44 > Tram in mungbean and Alankar > Pusa Bold > Sakha > PBM16 in mustard. The cultivars, Pusa Vishal and PDM54 (mungbean) and Alankar and Pusa Bold (mustard) showed lesser decreases in growth, photosynthetic and yield characteristics than the other cultivars, whereas, T44 and Tram (mungbean) and Sakha and PBM16 (mustard) suffered maximum decreases in growth and photosynthetic characteristics when subjected to 100mM NaCl (Tables 4-12,14-22). Thus, these cultivars, Pusa Vishal and PDM54 of mungbean and Alankar and Pusa Bold of mustard were categorized as tolerant and T44 and Tram of mungbean and Sakha and PBM16 of mustard as non-tolerant. As Pusa Vishal and Alankar cultivars were chosen for further study, these are referred to tolerant cultivars in the following pages. Similarly, the cultivars Tram and PBM16 are referred as non-tolerant cultivars. Moreover, tolerance index calculated for these cultivars also showed the tolerance behaviour of Pusa Vishal and Alankar and non-tolerant nature of Tram and PBM16 (Figure 1). The calculated tolerance index for plant dry mass of T44, Pusa Vishal, Tram and PDM54 was 47.02, 72.85, 41.94 and 71.39% in mungbean and 73.58, 70.78, 46.09 and 43.58% in Alankar, Pusa Bold, Sakha and PBM16 (mustard),

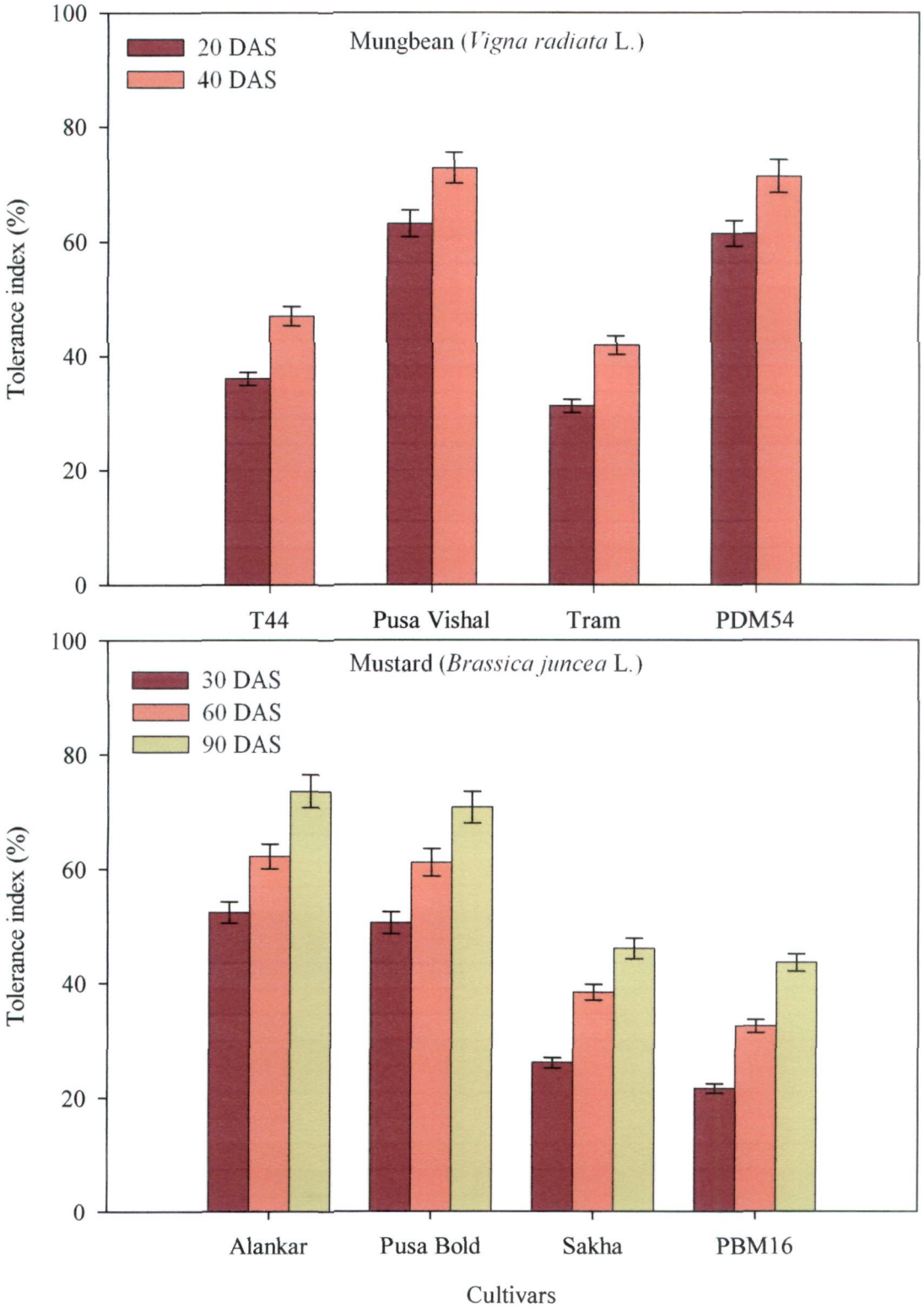


Figure 1: Tolerance index of four mungbean (Vigna radiata L.) and four mustard (Brassica juncea L.) cultivars exposed to 100mM NaCl. Tolerance index was calculated as percentage of plant dry mass obtained in 100mM NaCl and control. Data shown are mean ± S.E.

respectively. It may be assumed that the cultivars Pusa Vishal and Alankar possess inherent mechanisms to avoid the adverse effects of NaCl toxicity.

The salt tolerant cultivars Pusa Vishal and Alankar avoid the accumulation of toxic ions by an osmoregulatory process and ion accumulation (discussed in the section 'Introduction') and the absence of such mechanism in other cultivars affected the crop growth and productivity adversely. The following tolerance mechanisms in plants may operate under salt stress condition: (a) exclusion of salt followed by transport, compartmentation and/or secretion, (b) physiological, molecular and metabolic events that helps to tolerate the presence of salt at higher concentrations at the cellular level (c) morphological features such as rates of transpiration, stomatal closure, general inhibition of shoot growth with continued root growth.

Selective uptake and compartmentalisation of ions possessed by the crop cultivars are considered to be the basis of tolerance to salinity (Hedge and Joshi, 1974). An inverse relationship between salt tolerance and sodium accumulation found in plants suggested as an index of salt tolerance. It may be speculated that the tolerant cultivars of the reported study, Pusa Vishal of mungbean and Alankar of mustard possessed a combinations of mechanisms, salt exclusion, physiological and metabolic processes and inherent genetic characteristics, that helped them to show a lesser reduction in the characteristics and a higher tolerance index. An attempt was made to study these mechanisms in Experiments 3 and 4 (discussed later in separate headings).

In Experiments 1 and 2, the treatment of 100mM NaCl decreased fresh mass of both tolerant and non-tolerant cultivars (Tables 5,7,15,17). The poor growth performance of plants grown under saline conditions has been reported in the literature. Greenway and Munns (1980) and Cochorro *et al.* (1993) found that the decrease in growth was due to low water uptake and high internal salt concentrations or toxic effect of salts. Decrease in fresh mass has been

attributed to loss of water under stress conditions (Matsuda and Riazi, 1981; Binzel *et al.*, 1985; Cochorro *et al.*, 1993) and altered cell wall properties leading to wall loosening (Singh *et al.*, 1989). Legume is considered to be sensitive to salt stress, which affects its growth (Dua, 1992; Rao and Sharma, 1995), nodulation and nitrogenase activity (Elsheikh and Wood, 1990; Sheokand *et al.*, 1995), and photosynthesis and carbon metabolism in root nodules (Soussi *et al.*, 1998). Moreover, the deleterious effects of salinity on plant growth have been related to the decrease in osmotic potential of the growth medium, specific ion toxicity, nutritional imbalance and reduction in enzymatic and photosynthetic efficiency and other physiological disorders (Greenway and Munns, 1980; Ashraf *et al.*, 1991; Ashraf and Khan, 1993; Khan, 1993; Khan *et al.*, 1995). The results of the present study revealed that dry masses of all the cultivars decreased with the increasing salinity levels (Tables 6,8,10,16,18,20; Figures 2,5). The decrease in root and leaf dry masses due to salinity has been shown due to shrinkage of cell contents, reduced development and differentiation of vascular tissues (Strogonov, 1962), unbalanced nutrition (Hagazi *et al.*, 1995), damage of membrane and disturbed avoidance mechanism (Storey and Wyn Jones, 1978). Salinity affects on plants growth by decreasing the availability of water to the roots, due to the osmotic effect of external salt or by toxic effects of excessive salt accumulation within the plant (Munns, 1993; Munns *et al.*, 1995). Salinity stress also induces anatomical and morphological changes resulting in reduced growth (Seeman and Critchley, 1985). Exogenous NaCl is also known to inhibit cell division and enlargement (Wignarajah *et al.*, 1975). Salt accumulation in leaves causes premature senescence, reducing the supply of assimilates to the growing regions and, thus, decreasing plant growth (Munns *et al.*, 1995). In more sensitive cultivars, leaves are expected to die sooner because cells are unable to compartmentalize the salt in vacuoles to the same high degree as tolerant cultivars (Munns, 1993). Greenway and Munns (1980) suggested that when

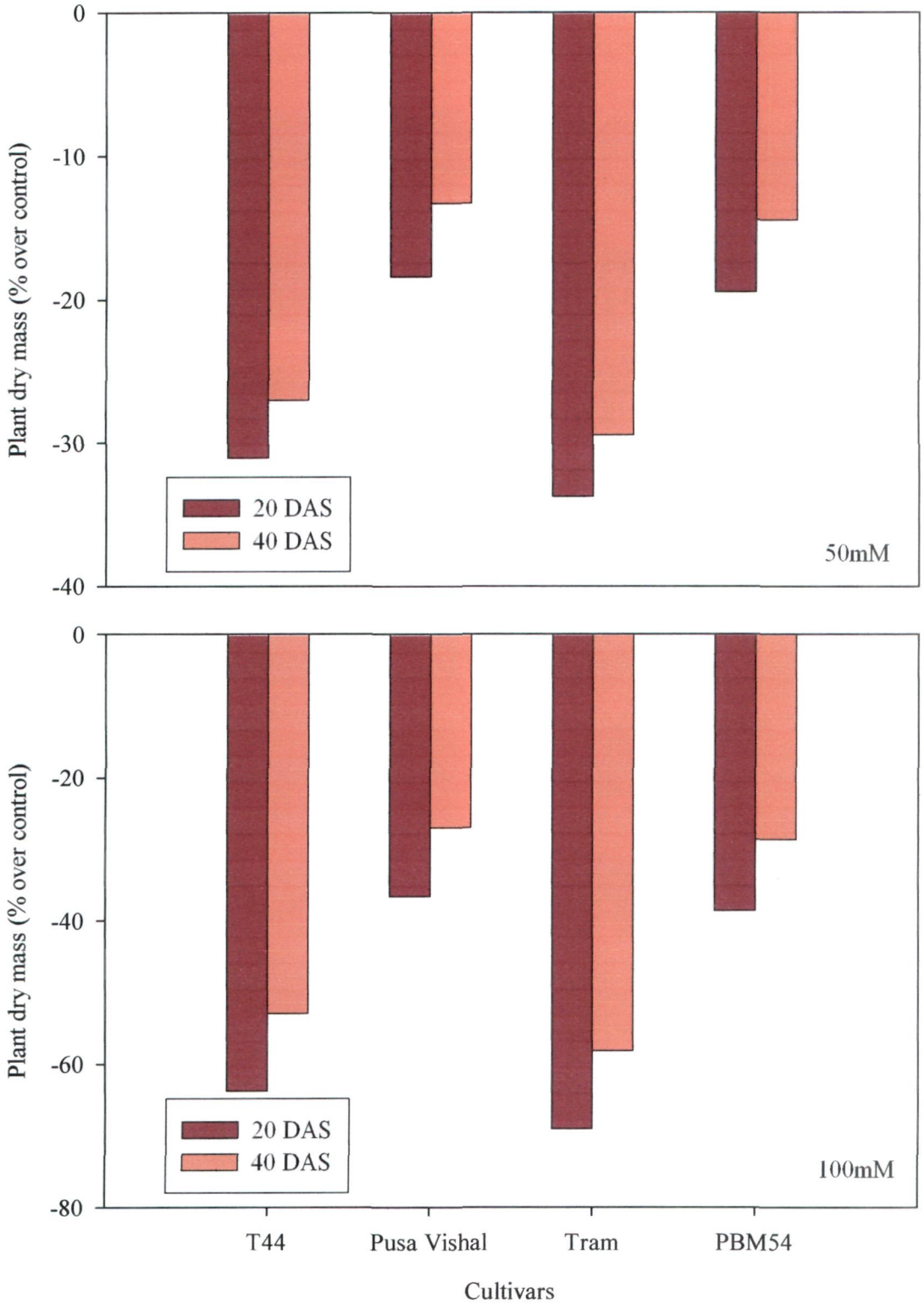


Figure 2: Per cent decrease in plant dry mass of mungbean (*Vigna radiata* L.) due to 50 and 100mM NaCl over control at 20 and 40 days after sowing (DAS).

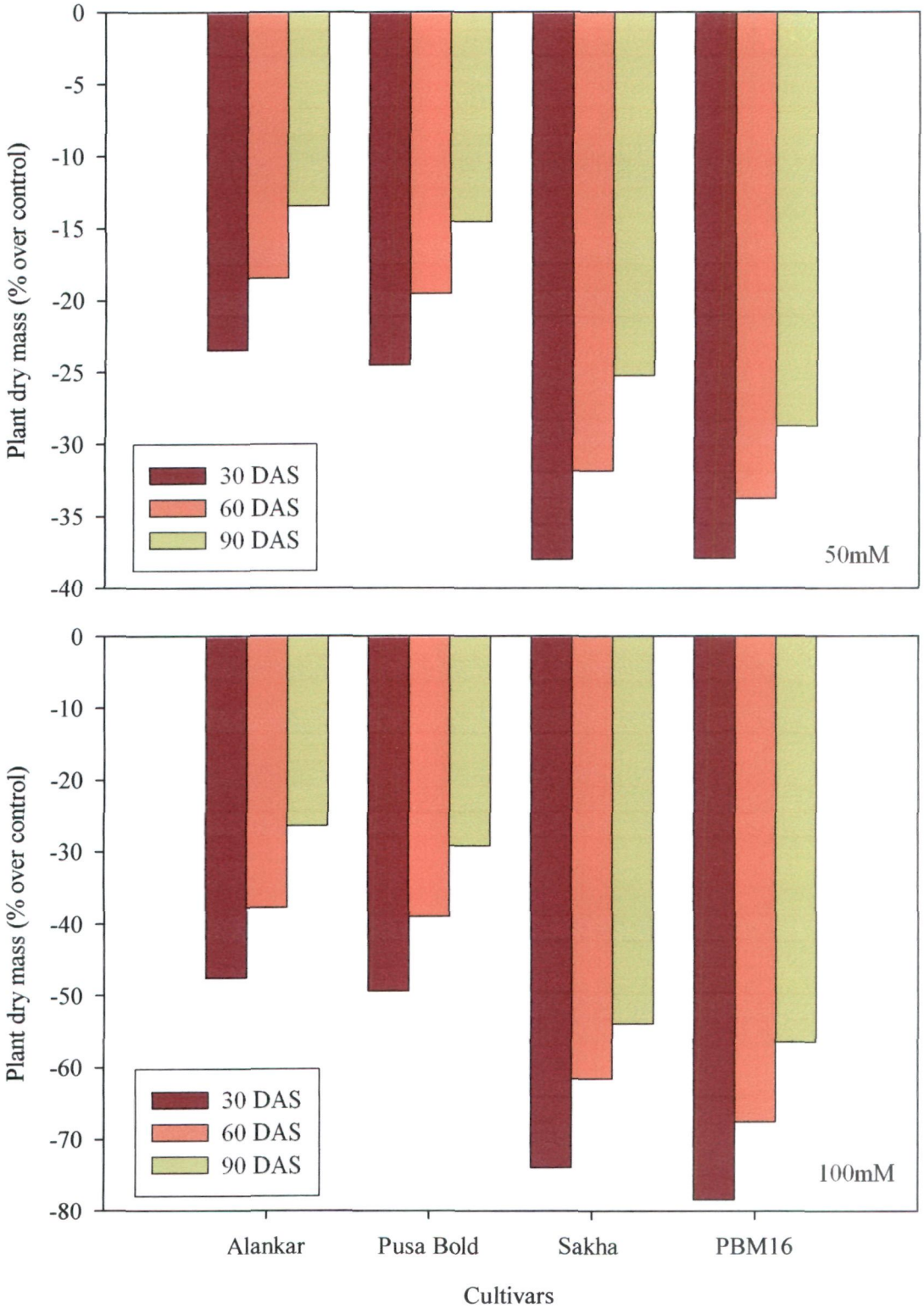


Figure 5: Per cent decrease in plant dry mass of mustard (*Brassica juncea* L.) due to 50 and 100mM NaCl over control at 30, 60 and 90 days after sowing (DAS).

plants were exposed to sodium chloride salinity, the Na^+ and Cl^- ions lowered the external water potential resulting in turgor reduction. As a result, these ions accumulated in the cytoplasm leading to the inhibition of plant growth and development. An increased concentration of chloride ions caused a continuous decline in growth rate of chick pea (*Cicer arietinum*) (Sangwan *et al.*, 1996). In *Raphanus sativus* total plant dry mass decreased at higher salinities (Marcelis and VanHooijdonk, 1999). Kurban *et al.* (1999) have reported that *Alhagi pseudoalhagi* (a leguminous plant), total plant dry mass increased at low salinity (50mM NaCl) but decreased at high salinity (100 and 200mM NaCl). Khan *et al.* (1999) have reported the fresh and dry mass of roots and shoot of *Halopyrum mocoronatum* (a perennial grass) responded favourably to 90mM NaCl and a further increase in the salinity concentration inhibited plant growth, resulting in plant death at 360mM NaCl. In a salt nonsecretor mangrove, *B. parviflora* the plant growth was optimal at 100mM NaCl whereas, further increase in NaCl concentration retarded plant growth and 500mM NaCl was found to be lethal, (Parida *et al.*, 2004a). In the case of salt secretor mangrove, *Aegiceras corniculatum* the plant tolerated upto 250mM NaCl and 300mM was found lethal (Mishra and Das, 2003). Greenway and Munns (1980) and Ng (1987) noticed increased growth at low salinity levels. This might be due to variable response of different plant species to salinity.

Salinity-induced decrease in leaf area has also been reported in the literature, as found in the present study (Tables 9,19). Ghoulam *et al.* (2002) showed that high NaCl concentrations caused a greater reduction in leaf area of sugarbeet. Grattan and Maas (1988) observed that high concentration of salinity (60, 80 and 120mM NaCl) decreased the growth of soybean. Maas *et al.* (1972) reported that the vegetative growth of bean was linearly depressed by salinity treatment, up to 100mM NaCl concentration. Increasing salt stress results in a considerable decrease in overall growth in tomato (Mohammad *et al.*, 1998), cotton (Meloni *et al.*, 2001), mungbean (Salim and Pitam, 1987;

Duong *et al.*, 1988; Gill, 1988; Patil *et al.*, 1992; Singh *et al.*, 1994; Raptan *et al.*, 2001), pea (Hernandez *et al.*, 1995), guava (AliDinar *et al.*, 1999), pepper (Chartzoulakis and Klapaki, 2000), and wheat (Arfan et al., 2007).

There are reports of concentration-dependent effects of salt stress on plant growth. NaCl has been shown to bring about a reduction in the overall growth and productivity of plants by perturbing the functioning of vital components of photosynthesis like PSI, PSII and the activity of Rubisco (Sivakumar *et al.*, 1998; Kawasaki *et al.*, 2001; Apse and Blumwald, 2002; Chen and Murata, 2002). Increasing concentration of NaCl in the present study also reduced the photosynthetic characteristics in all the cultivars of mungbean and mustard, and maximum reduction was observed with 100mM NaCl in Tram and PBM16. Photosynthetic characteristics of Pusa Vishal (mungbean) and Alankar (mustard) were higher compared to Tram (mungbean) and PBM16 (mustard) in the control and NaCl treatments. Inhibition of characteristics was more in Tram (mungbean) and PBM16 (mustard) than in Pusa Vishal (mungbean) and Alankar (mustard) (Tables 11-12,21-22; Figures 3,6). Salinity appeared to have an effect on two plant processes; water relations and ionic relations. During initial exposure to salinity, plants experienced water stress, which reduced leaf expansion. During long-term exposure to salinity, plants experienced ionic stress, which caused premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth (Cramer and Nowak, 1992). Reduced photosynthesis with increasing salinity has been shown either due to stomatal closure leading to a reduction in intercellular CO_2 partial pressure, or non-stomatal factors (Bethke and Drew, 1992). It is generally accepted that sink strength limits photosynthesis under several stress conditions (Seeman and Critchley, 1985). The decrease in photosynthesis may be considered as one of the important factors responsible for reduced plant growth and productivity under high salinity conditions (Ball *et al.*, 1987) as salinity has an influence on sink strength through changes in

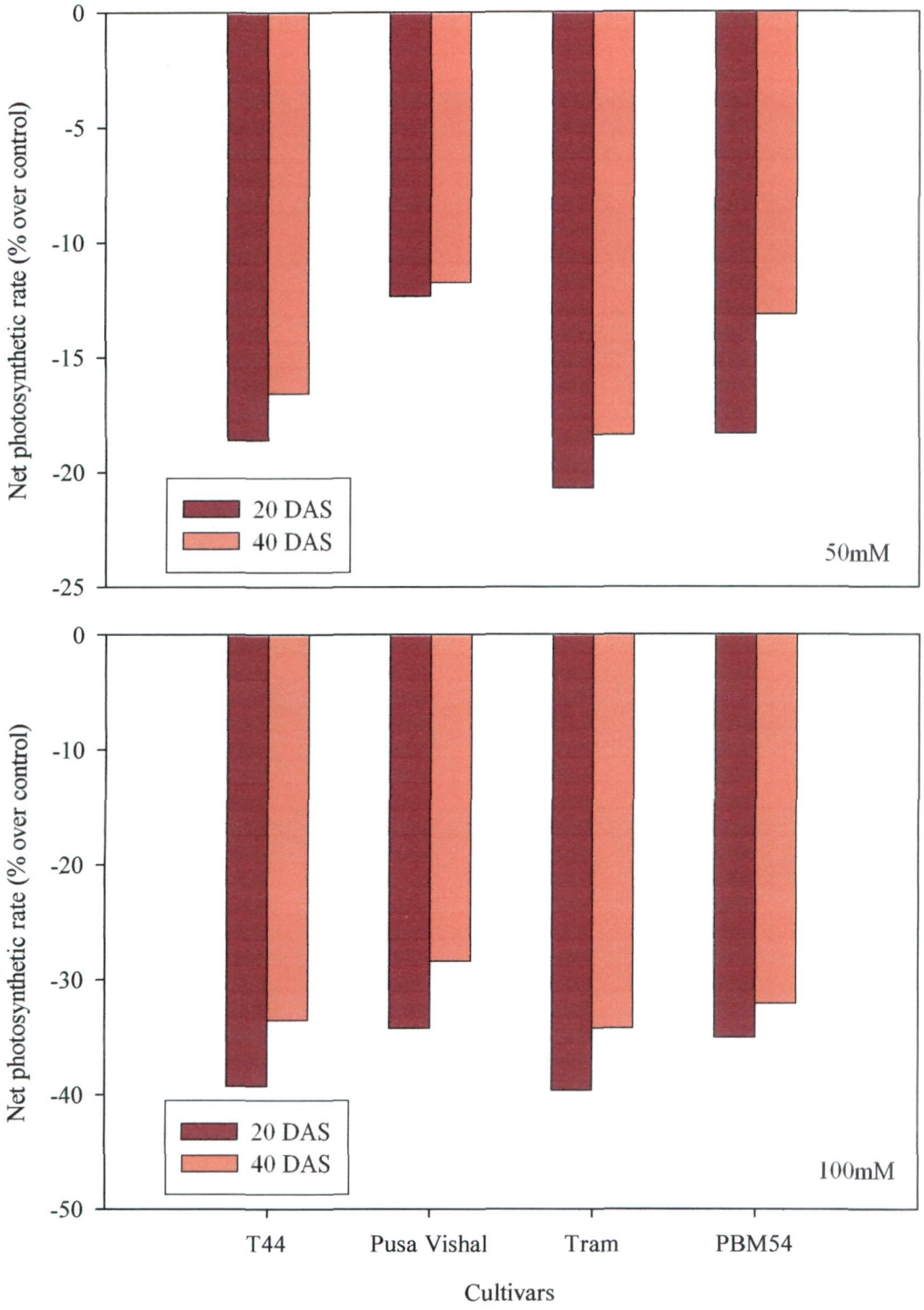


Figure 3: Per cent decrease in net photosynthetic rate of mungbean (*Vigna radiata* L.) due to 50 and 100mM NaCl over control at 20 and 40 days after sowing (DAS).

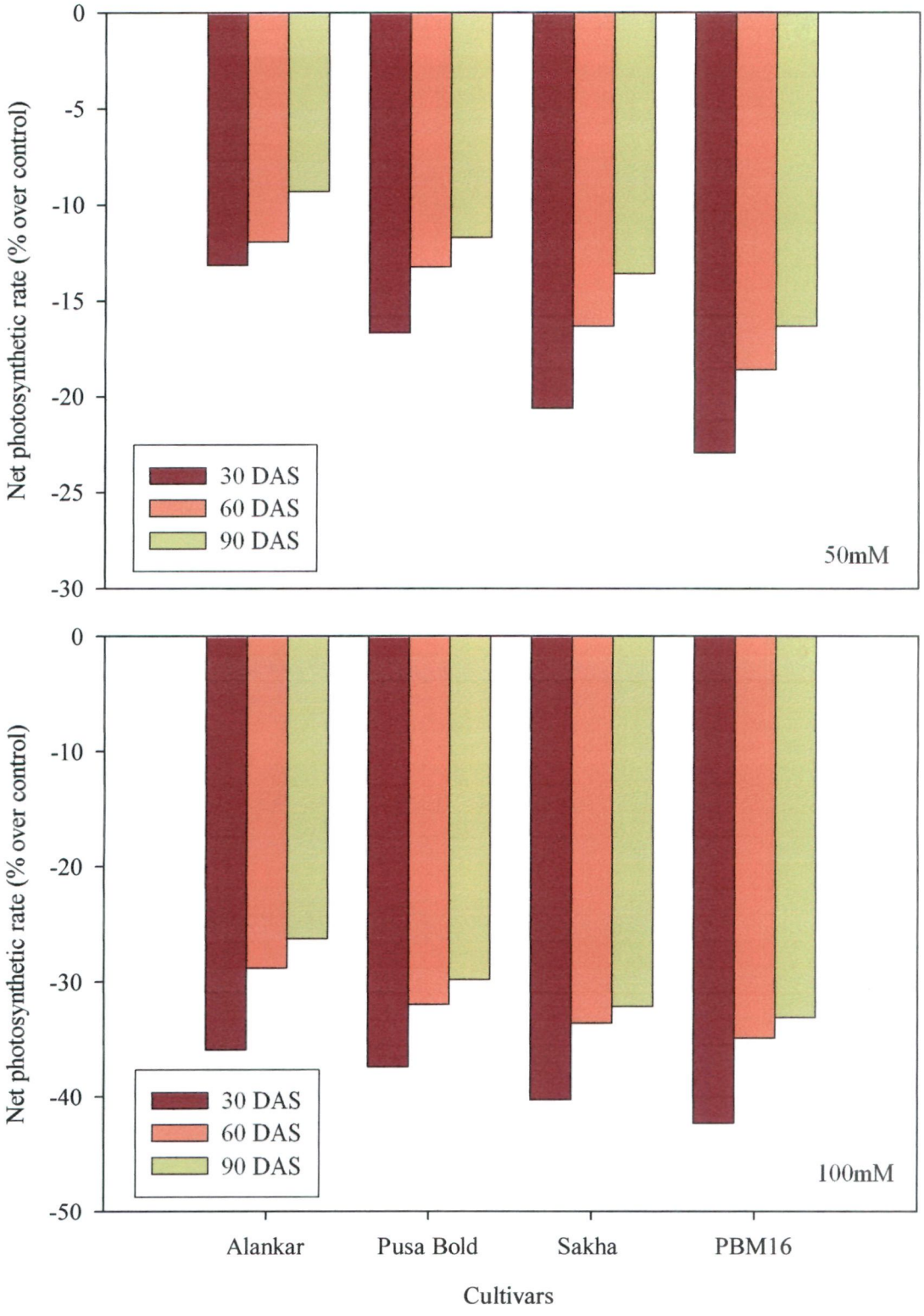


Figure 6: Per cent decrease in net photosynthetic rate of mustard (*Brassica juncea* L.) due to 50 and 100mM NaCl over control at 30, 60 and 90 days after sowing (DAS).

carbohydrate partitioning and accumulation (Paul and Foyer, 2001). In both the cultivars of the two crops, 100mM NaCl reduced stomatal conductance, the severe decrease being in non-tolerant cultivars. Decreases in photosynthetic rate are due to several factors : (1) dehydration of cell membranes which reduce their permeability to CO_2, (2) salt toxicity, (3) reduction of CO_2 supply because of closure of stomata, (4) enhanced senescence induced by salinity, (5) changes of enzyme activity induced by changes in cytoplasmic structure, and (6) negative feedback by reduced sink activity (Iyengar and Reddy, 1996). The decrease in net photosynthetic rate may also be attributed to the lowering of leaf water potential and a reduction in relative leaf water content, which results in loss of turgor and reduced net photosynthetic rate. The decline in net photosynthetic rate under salinity has been found closely related to the reduction in stomatal conductance (Nagy and Galiba, 1995; Lakshmi *et al.*, 1996), protein concentration (Sibole *et al.*, 1998) and photosynthetic pigment concentrations (Khan *et al.*, 1997). Yeo *et al.* (1985) also reported that the inhibition of net photosynthetic rate in rice by salinity was mediated by salt in the apoplast or low demand for photosynthates in the sink, viz. reproductive development, including grains (Karim *et al.*, 1993). Reduction in stomatal conductance caused a decrease in the amount of CO_2 uptake as a correlation between decreased stomatal conductance and reduced uptake of CO_2 has been found in salinity studies (Huang *et al.*, 1994; Marler and Zozor, 1996). This change in stomatal conductance limited the rate of CO_2 uptake and the rate of photosynthesis (Marler and Zozor, 1996). Salt stress causes either short-term or long-term effects on photosynthesis. The short-term effect occurs after a few hours or within 1 or 2d of the onset of exposure and this response is important as there is complete cessation of carbon assimilation within hours. The long-term effect occurs after several days of exposure to salt and reduction in carbon assimilation is due to salt accumulation in developing leaves (Munns and Termatt, 1986). There are also reports of suppression of photosynthesis upon

salt stress (Huang *et al.*, 1994; Chaudhuri and Choudhuri, 1997; Khavarinejad and Chaparzadeh, 1998; Soussi *et al.*, 1998; AliDinar *et al.*, 1999; Kao *et al.*, 2001; Romeroaranda *et al.*, 2001). The involvement of ABA in stomatal movement and photosynthesis regulation under salt stress has been reported. The ABA produced in response to salt stress decreases turgor in guard cells and limits the CO_2 available for photosynthesis (Leung *et al.*, 1994). Moreover, reduction of chloroplast stromal volume and generation of AOS under salt stress are also thought to play important roles in inhibiting photosynthesis (Price and Hendry, 1991). Photosynthetic activity decreases as water potential of leaves decreases (Iyengar and Reddy, 1996). High salt concentration in soil and water creates high osmotic potential, which reduces the availability of water to plants and affects photosynthesis. Decrease in water potential causes osmotic stress, which reversibly inactivates photosynthetic electron transport via shrinkage of intercellular space which is due to efflux of water through water channels in the plasma membrane (Allakhverdiev *et al.*, 2000a). Increase in osmotic potential under high salt conditions causes Na^+ ions to leak into the cytosol (Papageorgiou *et al.*, 1998) and inactivate both photosynthetic and respiratory electron transport (Allakhverdiev *et al.*, 1999).

Like growth and photosynthetic characteristics, yield and its attributing traits in mungbean and mustard were found decreased with the salt stress (Figures 4,7). The maximum effect was noted with 50mM NaCl as the plants grown with 100mM NaCl did not survive upto maturity, and therefore, no plants of both the crops were available under 100mM NaCl at harvest. The tolerant cultivars, Pusa Vishal of mungbean and Alankar of mustard exhibited lesser decrease in yield characteristics (like growth and photosynthetic characteristics) than the non-tolerant cultivars, Tram of mungbean and PBM16 of mustard (Tables 13,23). Cumulative factors like lower rate of assimilation and its translocation from shoots to sink, lower flowering percentage and increase in floret sterility might have contributed to the decrease in yield

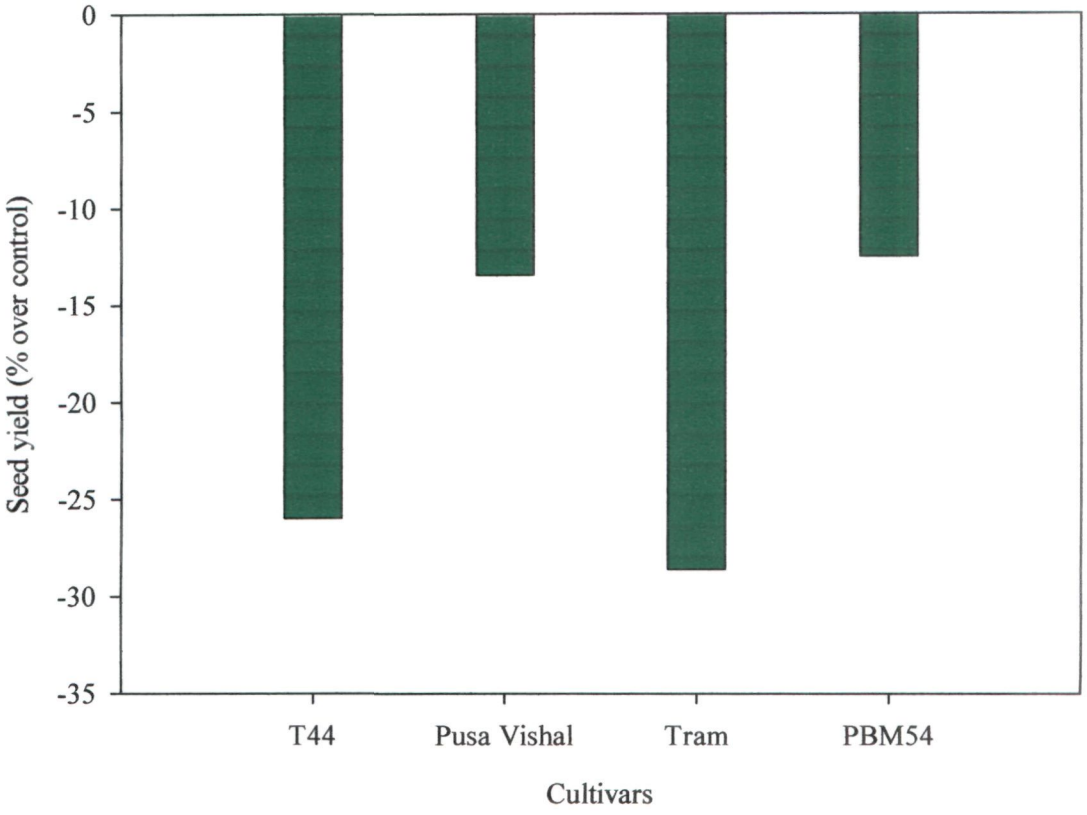


Figure 4: Per cent decrease in seed yield of mungbean (*Vigna radiata* L.) due to 50mM NaCl over control at harvest, i.e., 60 days after sowing (DAS).

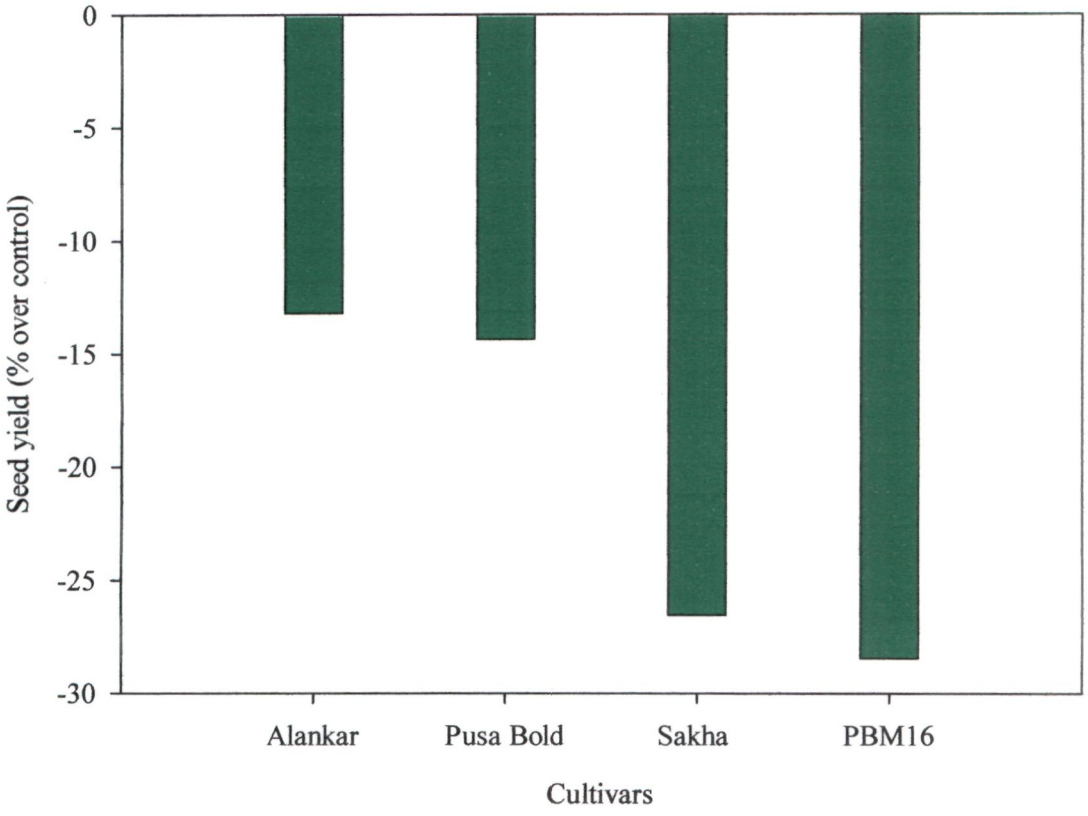


Figure 7: Per cent decrease in seed yield of mustard (*Brassica juncea* L.) due to 50mM NaCl over control at harvest, i.e., 120 days after sowing (DAS).

characteristics in both the crops. Salinity-induced reduction of dry matter accumulation in grains might also be due to the reduced rate of photosynthesis and the reduced ability to utilize photosynthates for growth. Salinity-induced reduction in yield has been reported in mungbean (*Phaseolus*) (Gill, 1990; Patil *et al.*, 1996; Raptan *et al.*, 2001), bajra (*Pennisetum*) (Sharma and Gill, 1992), *Cajanus* (Gill and Sharma, 1984) and rice (Greenway and Munns, 1980; Yeo and Flowers, 1982). A contrasting report of Fauzia *et al.* (1988) showed that pods per plant and seeds per pod were not significantly affected up to the salinity level of 7.5dsm^{-1} in mungbean. The different result might be due to a different experimental condition.

In the present study, the severity of salinity effects on the growth, photosynthetic and yield characteristics was higher in the salt sensitive cultivars Tram and PBM16, indicating that NaCl had negative influence on plants performance. The tolerance of Pusa Vishal (mungbean) and Alankar (mustard) appeared to be associated with the maintenance of higher growth, photosynthetic and yield characteristics under stress conditions. In this study it was demonstrated that salinity inhibited the performance of mungbean and mustard. The cultivars Pusa Vishal (mungbean) and Alankar (mustard) tolerated salinity-induced effects to a higher degree. Therefore, the cultivars, Pusa Vishal and Alankar showed higher tolerance than Tram and PBM16 in terms of growth, photosynthetic and yield characteristics.

5.3 Effect of Salicylic Acid on Cultivars Differing in Salt Tolerance

Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. Biochemical pathways leading to improve salt tolerance may act additively or synergistically (Iyengar and Reddy, 1996). Biochemical strategies that plants adopt include (i) selective accumulation or exclusion of ions, (ii) control of ion uptake by roots and transport into leaves, (iii) compartmentalization of ions at the cellular and whole plant levels, (iv) synthesis of compatible solutes, (v) change in photosynthetic pathway, (vi)

alteration in membrane structure, (vii) induction of antioxidative enzymes, and (viii) induction of plant hormones.

Of all the factors listed above, the effect of plant hormones has been considered of primary importance as the hormone may affect other factors responsible for tolerance. Concerted attempts have been made to mitigate the harmful effects of salinity by the application of plant growth regulators (Darra *et al.*, 1973; Datta *et al.*, 1998; Khan, 2004).

Salicylic acid is a naturally occurring phenol acting as plant growth regulator, and phenols and phenolic compounds are of considerable importance in the regulation of plant growth and metabolism (Vendrig and Buffel, 1961; Jindal and Singh, 1975). Evidences are accumulating to confirm that SA and other derivatives such as acetyl salicylic acid or aspirin (2-hydroxy 3-methyl benzoic acid, sulfo salicylic acid (2-hydroxy 5-sulfo salicylic acid) could induce tolerance in plants to a variety of abiotic stresses (Senaratna *et al.*, 2003). Exogenous application of SA, either by direct injection or by spraying have been reported to cause a multitude of effects on the morphology and physiology of plants (Raskin, 1992; Pierpoint, 1994; Pancheva *et al.*, 1996).

In view of the importance of SA in inducing tolerance against abiotic stresses, the study reported in the thesis was conducted to improve our understanding on the involvement of SA in tolerance mechanism of mungbean and mustard plants under salinity stress. For this, two cultivars of mungbean and mustard differing in salt tolerance were selected after screening on the basis of growth, photosynthetic and yield characteristics (Experiments 1 and 2). These cultivars were treated with SA concentrations and morphological, physiological and biochemical characteristics were studied to find the alleviating effect of SA against salt stress. The results of the study have been discussed in the following pages with relevant information available in the literature and other possible mechanisms have been discussed. Some of the

important inference drawn from the study that could help in alleviating salt stress has been proposed as future programme of study.

5.3.1 Growth characteristics

In Experiments 3 and 4, growth characteristics decreased significantly with 50mM NaCl in tolerant and non-tolerant cultivars but more adverse effects of salinity were found on Tram (mungbean) and PBM16 (mustard) (Tables 24-30,47-53) (discussed in the section 'Comparison of Cultivars Performance under Salinity Stress'). Application of 0.5mM SA increased growth characteristics of tolerant and non-tolerant cultivars grown under saline and non-saline conditions (Tables 24-30,47-53; Figures 8,11). Non-salinized plants maintained higher growth characteristics indicating adverse effect of the NaCl. In Alankar (mustard), the growth characteristics increased over control with the application of 0.5mM SA on plants treated with 50mM NaCl (Tables 47-53). Pretreatment of banana seedlings with 0.5m mol/L SA in hydroponic solution significantly decreased the area of wilting leaf areas, or inhibited leakage of electrolytes throughout cell membranes during chilling stress, indicating that it enhanced the chilling tolerance of banana plants.

Presowing treatment of wheat seeds with SA contributes to the increase in the resistance of plants to stress factors of environment (Sakhabutdinova *et al.*, 2003). Shoot growth was increased with the three concentrations of SA (10^{-2}M, 10^{-4}M and 10^{-8}M) used and an average increase of 23% and 20% in plant height was observed under greenhouse conditions and field experiments, respectively. The three concentrations of SA significantly increased root length; e.g. an increase of 45% in relation to control was found under greenhouse conditions with 10^{-8}M treatment. The effect of SA on root growth was greater in pot-field experiment with an increase of up to 100% over controls detected in 10^{-2} and 10^{-8}M treatments (Gutierrez-Coronado *et al.*, 1998). SA could be involved in the regulation of cell enlargement and division in synergy with other substances such as auxin, which is recognized to regulate

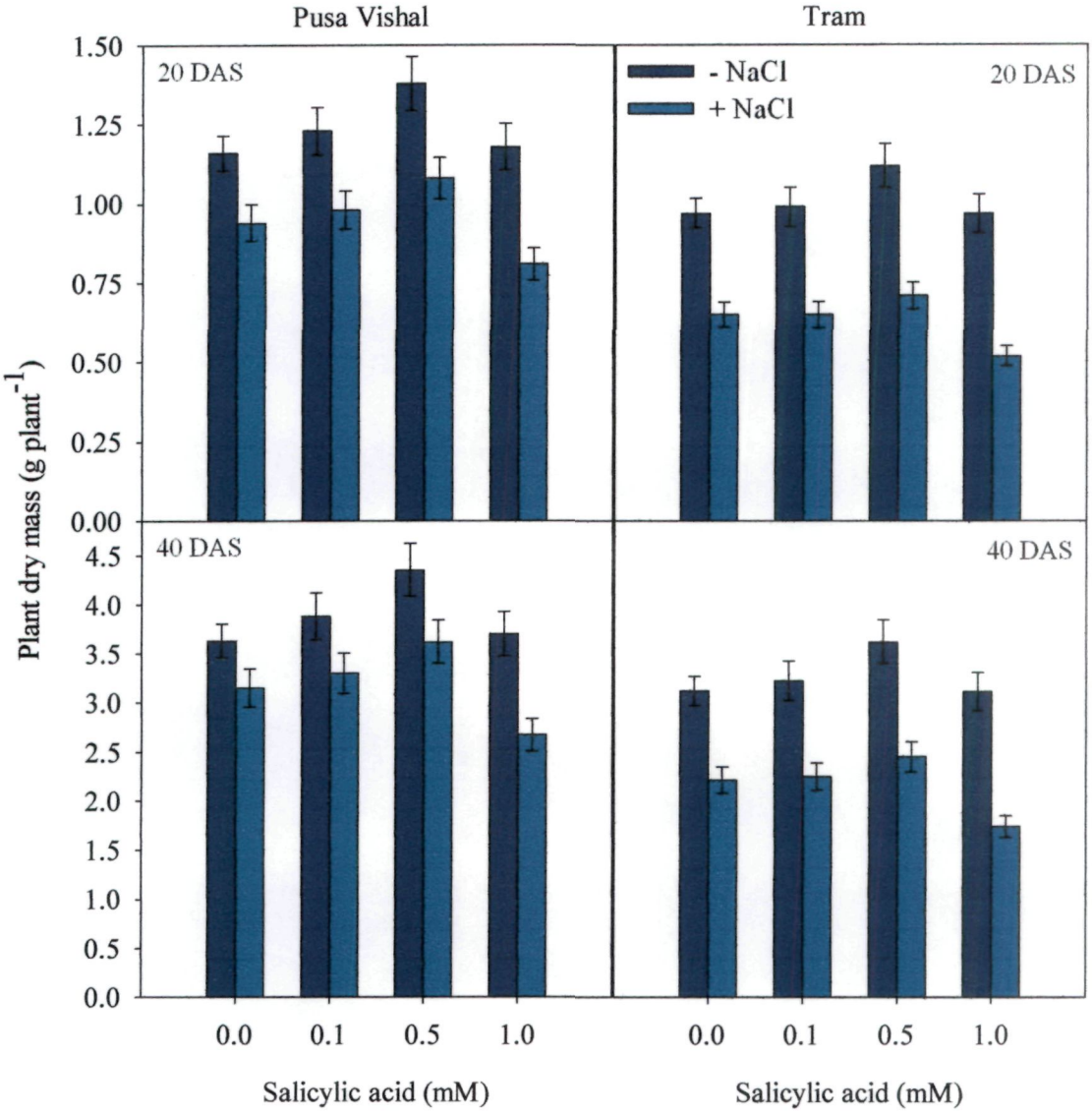


Figure 8: Effect of increasing concentrations of SA and salinity stress on plant dry mass of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) at 20 and 40 days after sowing (DAS). Data shown are mean ± S.E.

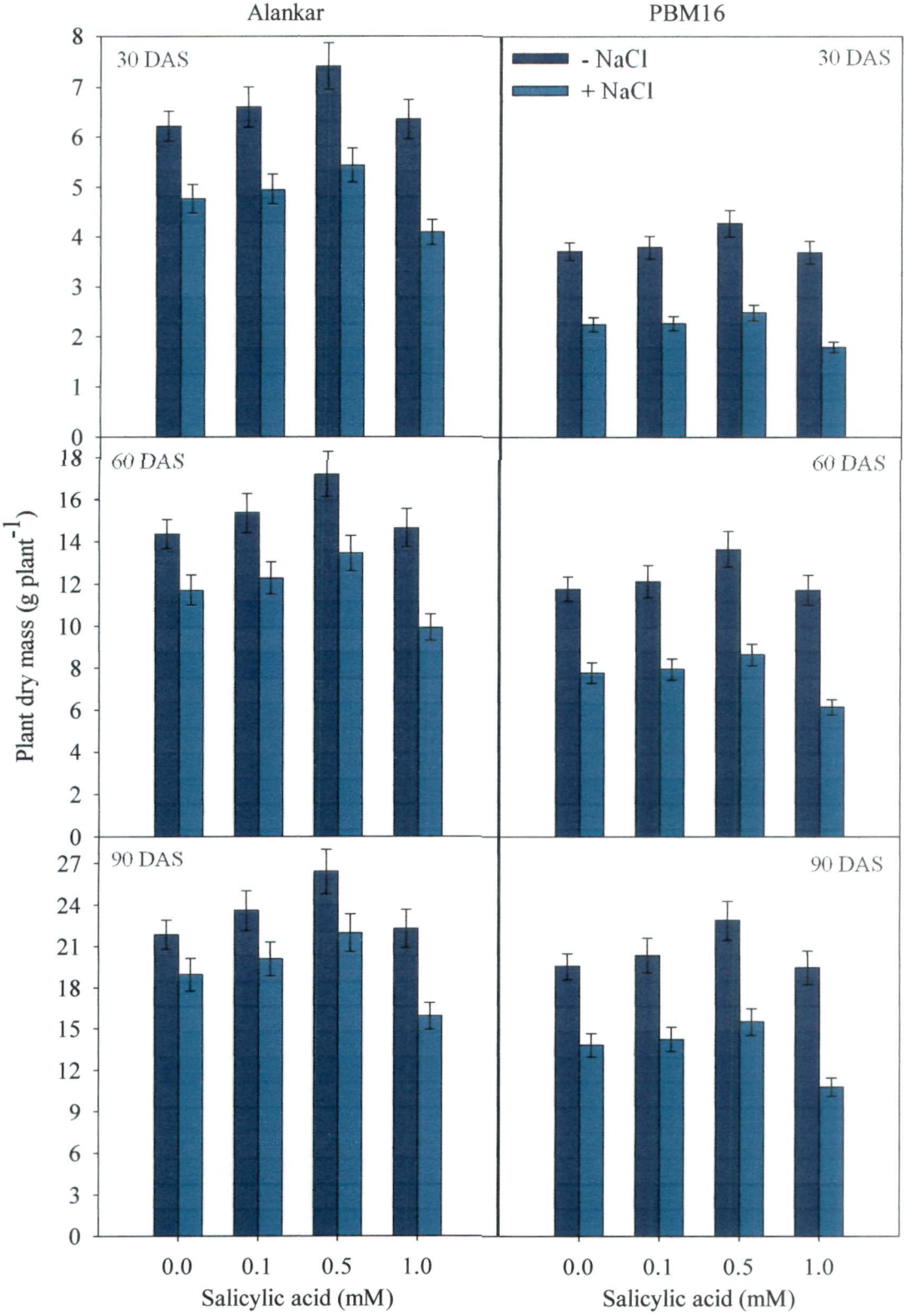


Figure 11: Effect of increasing concentrations of SA and salinity stress on plant dry mass of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS). Data shown are mean ± S.E.

cell enlargement and division during root formation (Singh and Kaur, 1980; Kling and Meyer, 1983; Li and Li, 1995). Singh (1993) found that SA stimulated root formation in young shoots of ornamental plants and Li and Li (1995) reported the formation of adventitious roots on hypocotyl cuttings of mungbean. Khodary (2004) reported that SA increased fresh and dry mass of shoot and roots of salt-stressed maize plants. Gutierrez-Coronado *et al.* (1998) also reported a similar increase in the growth of shoots and roots of soybean plants in response to SA treatment. Dhaliwal *et al.* (1997) and Zhou *et al.* (1999) also indicate that SA increases leaf area in sugarcane plants.

5.3.2 Photosynthetic characteristics

Photosynthetic characteristics of tolerant and non-tolerant cultivars decreased with the application of 50mM NaCl. The effect of 0.5mM SA application on the photosynthetic characteristics of both tolerant (Pusa Vishal and Alankar) and non-tolerant (Tram and PBM16) cultivars was positive under non-saline condition and ameliorative under salinity stress (Tables 31-36,54-59; Figures 9,12). Salt stress has been found to affect photosynthetic components, such as enzymes, chlorophylls, and carotenoids, which depend on the severity and duration of the stress (Lakshmi *et al.*, 1996; Misra *et al.*, 1997) and on plant species (Dubey, 1994). Several plant functions are inhibited due to salt stress leading to a cumulative adverse effect on photosynthesis, e.g., decrease in chlorophyll concentration in salinized plants has been attributed to the increased activity of chlorophyllase, a chlorophyll-degrading enzyme (Reddy and Vora, 1986). Additionally, sodium accumulation in leaves has also been found to adversely affect chlorophyll synthesis (Yeo and Flowers, 1983). At high salinity stress sodium and chloride loosened the binding between chlorophyll and chloroplast protein and as a result chlorophyll is destroyed (Afria *et al.*, 1998). The reduction in chlorophyll content under salinity has also been reported earlier in several crops (Lapina and Popov, 1970; Varshney,

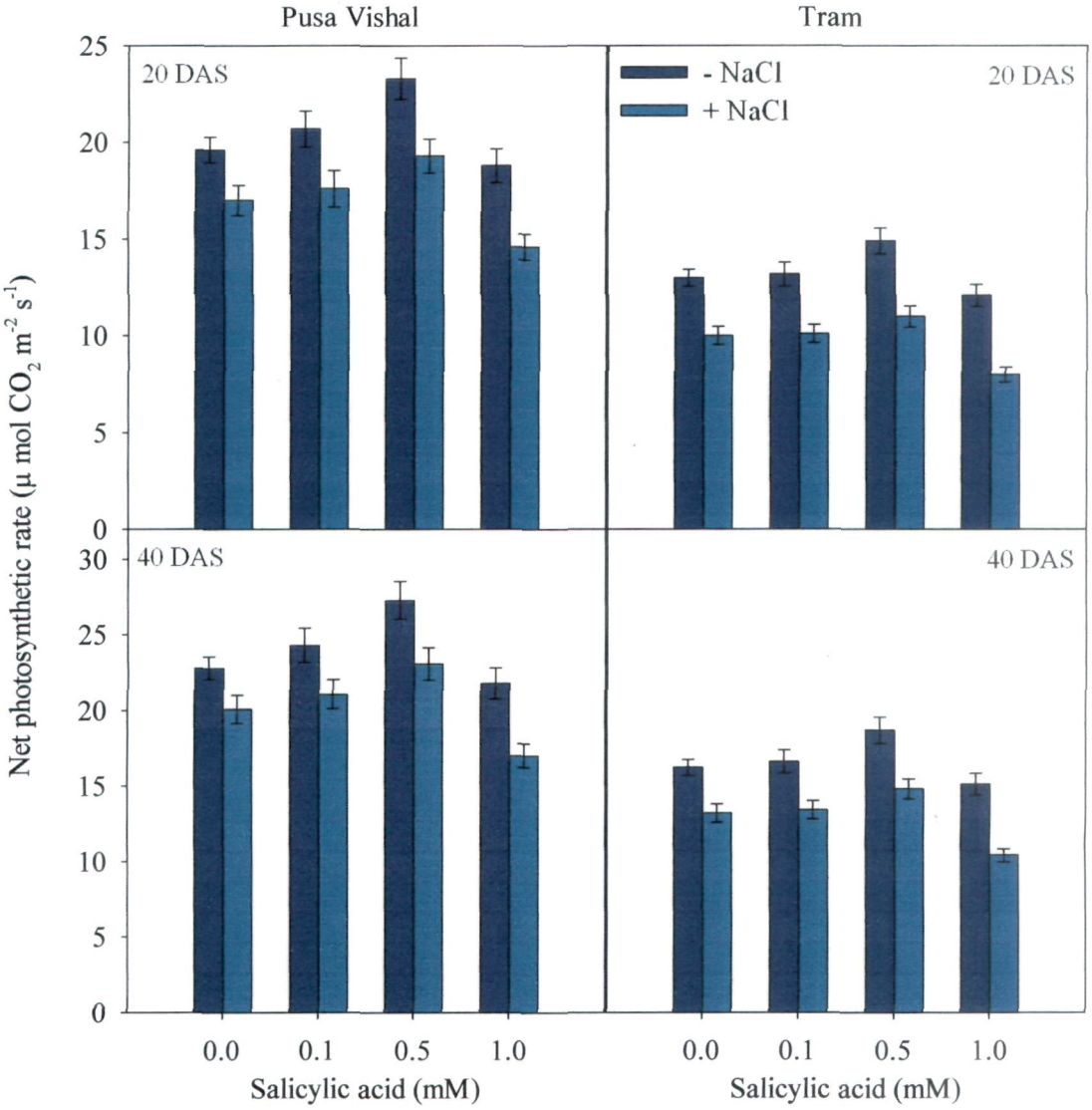


Figure 9: Effect of increasing concentrations of SA and salinity stress on net photosynthetic rate of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) at 20 and 40 days after sowing (DAS). Data shown are mean ± S.E.

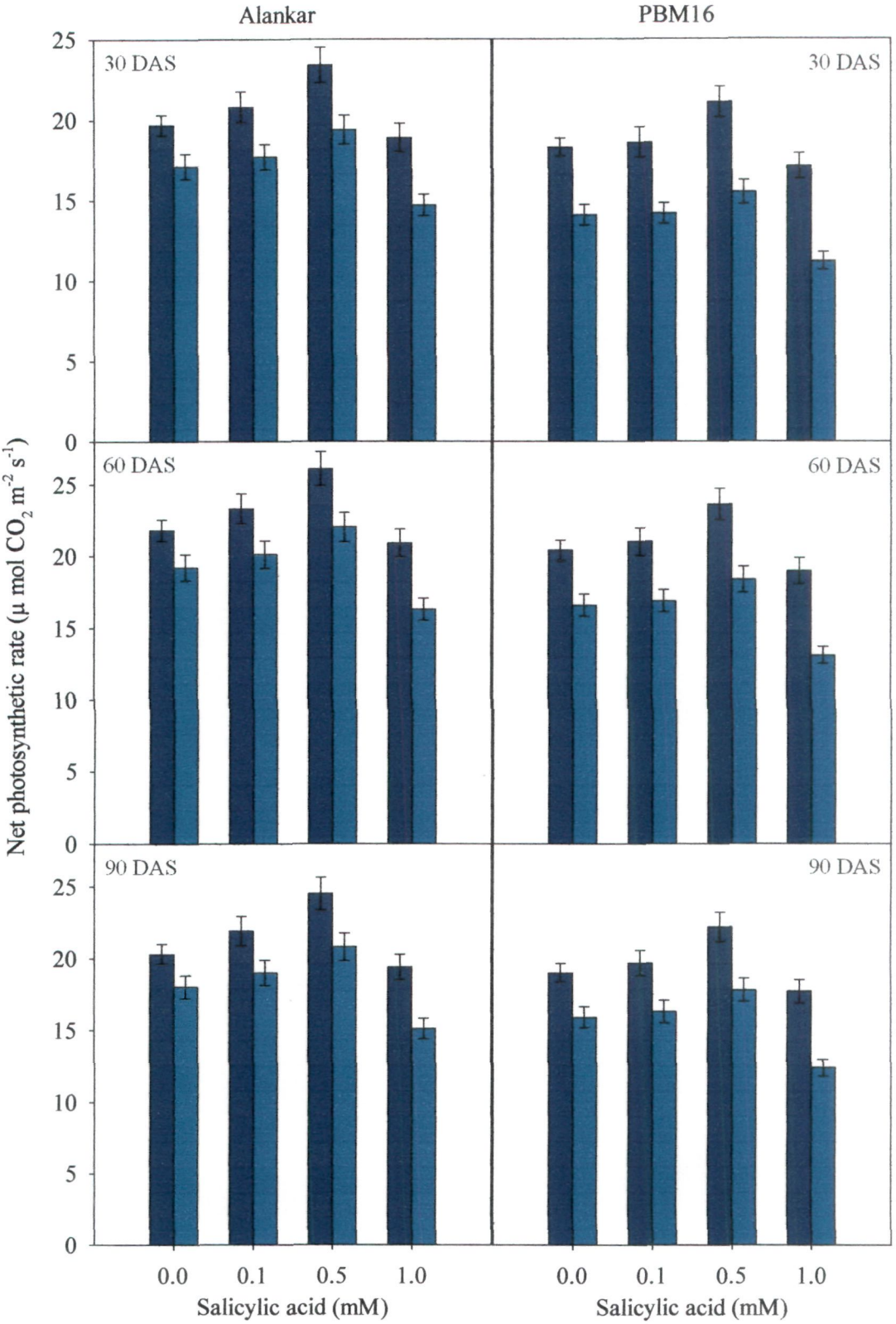


Figure 12: Effect of increasing concentrations of SA and salinity stress on net photosynthetic rate of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS). Data shown are mean ± S.E.

1980; Ball *et al.*, 1987; Garg, 1987; Lahiri *et al.*, 1987; Akhavan-Kharzian *et al.*, 1991).

In higher plants, carotenoid protects the photosynthetic apparatus from excess photons and oxidative stress, which are generated under stress (Siefermann-Harms, 1987; Panda and Biswal, 1989; Srichandan *et al.*, 1989; Demmig-Adams, 1990; Young, 1991). Carotenoids are thought to be involved in the protection against stress as they undergo degradation and formation of zeaxanthins, which protects from photoinhibition (Sharma and Hall, 1991). In general, salt stress decreases chlorophyll and carotenoid contents, but report of unaltered carotenoid content in alfalfa is available (Khavarinejad and Chaparzadeh, 1998). Khavarinejad and Mostofi (1998), Parida *et al.* (2002) have reported decreases in chlorophyll a+b, chlorophyll a, chlorophyll b and β-carotene by NaCl stress. Seawater treatments decrease carotenoids in *Zea mays* seedlings and induce reduction in chlorophyll and net photosynthetic rate (El-Shihaby *et al.*, 2002). In cyanobacterium, *Spirulina platensis*, a decrease in the phytocyanin/chlorophyll ratio and no significant change in the carotenoid/chlorophyll ratio have been observed under salt stress (Lu and Vonshak, 1999).

The results obtained in Experiments 3 and 4 also show decrease in the contents of chlorophyll and carotenoids due to 50mM NaCl. It was also found that such decreases could be reduced by the application of 0.5mM SA. Application of 0.5mM SA increased photosynthetic characteristics of mungbean and mustard. In both tolerant and non-tolerant cultivars, the increases were greater under non-saline (control) conditions. At 40DAS, the treatment of 0.5mM SA not only reduced the adverse effects of 50mM NaCl but also increased the characteristics in comparison to the respective control in tolerant mungbean cultivar Pusa Vishal plants. At 60 and 90DAS, the treatment of 0.5mM SA on tolerant mustard cultivar Alankar overcome the NaCl effect and increased the characteristics of plant grown under 50mM NaCl (Tables 54-

59). The alleviating effect of SA on photosynthesis could be attributed to the protection of chlorophyll against degradation, increase in carotenoid content and favourable effect on photosynthetic enzymes.

A concentration of 10mg kg^{-1} SA has been found to increase total chlorophyll content, and further increase in the SA concentration decreased the chlorophyll (Moharekar *et al.*, 2003). Pretreatment of plants with SA for 24h before paraquat application caused a protection against paraquat-induced chlorophyll loss (Popova *et al.*, 2003). Contrarily, reduction in chlorophyll content in barley leaves following the application of SA was found by Pancheva *et al.* (1996). Anandhi and Ramanujam (1997) also found decline in chlorophyll content of *Vigna mungo*. This might be due to use of high concentration of SA. Popova *et al.* (2003) reported that dark-treated barley seedlings with 500μM SA did not show loss in chlorophyll contents.

The photosynthetic pigment, carotenoid has been attributed to the cause of protection against stress. In the present study, tolerant cultivars of mungbean and mustard, Pusa Vishal and Alankar, showed an increase of 18.39 and 20.00% at 20 and 40DAS and 19.35, 19.69 and 20.90% increase at 30, 60 and 90DAS in carotenoid content due to 0.5mM SA application. Although high concentratioin (1.0mM SA) inhibited carotenoid formation. As mentioned earlier, the response of a plant to SA depends on the sensitivity of plants to SA. In *Arabidopsis thaliana*, 1mM SA caused an increase in carotenoid content, whereas 5mM SA caused a decrease in the carotenoid content in comparison to the control (Rao *et al.*, 1997). Sinha *et al.* (1993) pointed out that chlorophyll and carotenoid contents of maize leaves increased upon treatment with SA. The reduction in salt stress-induced loss in chlorophyll a and carotenoid contents by the application of SA in the presence of 100mM Na^+ has been reported by Tari *et al.* (2002). Similarly, Khodary (2004) has found higher values of pigment concentration than those of control or salinity-treated plants due to SA application. Comparing the plant types, Moharekar *et al.* (2003) found that total

chlorophyll content was higher in wheat than in moong and total carotenoid content was higher in moong than in wheat. From this study it was concluded that lower chlorophyll content in moong might induce greater oxidative stress than in wheat. The results of the reported study also show that tolerant cultivars of mungbean and mustard showed higher photosynthetic pigments and lesser oxidative stress. SA application has been found to increase photosynthesis under normal and stress conditions due to its effect on photosynthetic pigments and enzymes of the Calvin cycle. Liu *et al.* (1999) reported that SA enhanced the photosynthetic ability of apple leaves which was attributed to the stimulatory effect of SA on Rubisco activity and pigment contents. In soybean plants, treatment of SA increased photosynthetic rate (Zhao *et al.*, 1995). Treatment of barley seedlings with 500μM SA for 24h in the dark followed by 6h light exposure did not damage to photosynthesis (Popova *et al.*, 2003). In my experiments on mungbean and mustard, the treatment of plants with 0.5mM SA caused greatest increase in the rate of photosynthesis. This SA-enhanced photosynthesis under salt stress is attributed to the increase in the photosynthetic pigments and carbonic anhydrase activity, an enzyme responsible for reversible hydration of CO_2. The effect of SA on the carbonic anhydrase activity of salt-stressed mungbean and mustard has not been reported earlier, however, earlier it has been shown by Khan *et al.* (1996) from our laboratory that gibberellic acid enhanced the carbonic anhydrase activity in mustard.

The effect of SA on photosynthesis involved stomatal and non-stomatal limitation in both the crops. The increase in stomatal conductance and intercellular CO_2 concentration in response to 0.5mM SA application suggests stomatal effects on photosynthesis. Moreover, SA-enhanced carbonic anhydrase activity indicates involvement of non-stomatal effects on photosynthesis. Additionally, it may be said that SA increased the rate of ethylene release and application of 0.5mM SA increased ethylene concentration

that enhanced maximum photosynthesis. Studies have shown that ethylene affects photosynthesis as a result of variation in stomatal conductance (Gunderson and Taylor, 1991;Kamaluddin and Zwiazek, 2002; Khan, 2004). Also, the increased allocation of nutrients, nitrogen, phosphorus, potassium and calcium to leaf resulting from SA applicatioin (discussed in the next section) helped in stimulating photosynthesis of SA treated plants. The allocation of nitrogen to the photosynthetic machinery might have stimulated the rate of photosynthesis as the role of nitrogen in photosynthesis has been well described (Marschner, 1995).

5.3.3 Biochemical characteristics

Salt tolerance is expected to involve mechanisms which limit the accumulation of toxic ions in different plant parts. The involvement of ionic and organic solute synthesis and its accumulation also play a key factor in salt tolerance (Mass and Nieman, 1977).

Salinity toxicity is caused by the ionic intoxication of the cytoplasm (Niu *et al.*, 1995; Yeo, 1998). Salt sensitive and salt tolerant plants differ in the sensitivity of cytoplasmic metabolic processes and thus ionic intoxication. Salt tolerant plants possess mechanisms that avoid the accumulation of cytoplasmic Na^+.Application of SA proved effective in ameliorating the adverse effects of salinity on biochemical characteristics. The concentrations of sodium and chloride increased significantly with the salinity treatment. In this study, the salt sensitive plants (Tram and PBM16) accumulated more sodium than the tolerant plants (Pusa Vishal and Alankar) (Tables 37,60) due to the difference in the absorption capacity. The difference in sodium absorption capacity of plants has been reported in maize (Yeo *et al.*, 1977), rice (Yeo and Flowers, 1984b) and wheat (Joshi *et al.*, 1985; Ralph and Epstein, 1986). The application of SA on both the cultivars had significant effect in restricting the high accumulation of sodium and chloride compared with control under normal and saline conditions. The application of 0.5mM SA proved most effective in

reducing sodium and chloride concentrations in the plants (Tables 37-38,60-61). Increased treatment of NaCl increased the Na^+ and Cl^- levels in a number of plants (Gadallah, 1999; Khan *et al.*, 1999, 2000a; Khan, 2001).

Application of SA consistently maintained sodium and chloride concentrations. The effects of 50mM NaCl were reverved with the application of 0.5mM SA. The application of 0.5mM SA on plants fed with 50mM NaCl decreased the concentrations of sodium and chloride in tolerant and non-tolerant cultivars in comparison to the control (Tables 37-38,60,61). The positive effect of 0.5mM SA was found as it decreased sodium and chloride concentrations under both saline and non-saline conditions. Decreasing levels of Na^+ and Cl^- ions in saline condition explains the ameliorating effect of SA, probably due to the protective role of SA on membranes which regulates ion uptake as suggested by Mishra and Choudhuri (1999) and Alpaslan and Gunes (2001). The application of SA has also been found by other authors to decrease Na^+ and Cl^- concentrations significantly in salinity stress conditions (Gunes *et al.*, 2005, 2007). The study of Tari *et al.* (2002) showed that SA-pretreated leaves under salt stress increased water potential of the leaf tissues. Tari *et al.* (2002) reported that excess of sodium ions did not cause the well-known symptoms of salt stress in SA-treated tomato plants. In case of salinity stress, the treatment of 10^{-7}-10^{-4}M SA enhanced the translocation of Na^+ from roots to shoots but decreased the Na^+/K ratio in the roots and increased it significantly in the leaves of tomato plants. Szepesi *et al.* (2005) argued that Na^+ accumulated in the leaf tissues and functioned as an inorganic osmolyte resulting in an increased water potential and water content. Al-Hakimi and Hamada (2001) also showed the similar results that SA decreased Na concentration of wheat shoot and root tissues under salinity. The concentrations of nitrogen, phosphorus, potassium and calcium also influence tolerance of plants to salinity. Nitrogen, phosphorus, potassium and calcium concentrations were found decreased significantly with salinity (Tables 39-42,62-65), but they

increased with the application of 0.5mM SA under both normal and saline conditions in both tolerant and non-tolerant cultivars (Tables 39-42,62-65).

Nitrogen promotes growth and development of plants by increasing nitrogenous metabolites (Marschner, 1995). The uptake of nitrogen helps in the synthesis of nitrate into organic nitrogenous compounds and induces tolerance (Lapina, 1967). Nitrogen metabolism is adversely affected by salt stress in plants. A decrease in total nitrogen content due to salinity stress has been reported by Paliwal and Maliwal (1980) in sorghum and green gram, Karadge (1981) in *Portulaca oleracea*, He and Cramer (1992) in three *Brassica* species, and Parida and Das (2004) in leaves of *B. Parviflora*. Hafeez *et al.* (1988), Patil *et al.* (1995), and Singh *et al.* (1994) showed that nitrogen uptake decreased due to salinity in mungbean. Helal *et al.* (1975) showed that young barley plants showed impaired uptake of labeled nitrogen under salinity.

Saline conditions influence Na^+ and K^+ content, ionic imbalance in plant system and increased Na^+/K^+ ratio (Sharma, 1990; Shannon and Noble, 1995; Datta *et al.*, 1996). Salinity decreased K^+ and increased Na^+ and Cl^- contents in roots, stems and leaves of wheat (Chhipa and Lal, 1993; Khan *et al.*, 1999), rice (Prakash and Prathapasenan, 1988), and maize (Datta *et al.*, 1996) but the application of different growth regulators has been found to increase K^+ and decrease Na^+ and Cl^- contents under salt stress. Na^+-induced depletion of tissue K^+ has been cited as a contributor to salinity toxicity (Ben-Hayyim *et al.*, 1987; Nakamura *et al.*, 1990), but the effect of Na^+ on tissue K^+ has been variable. Supplementation of rooting media with NaCl may cause reductions of tissue K^+ concentrations (Kingsbury and Epstein, 1986; Ben-Hayyim *et al.*, 1987; Nakamura *et al.*, 1990) or elevations of tissue K^+ concentrations (Boursier and Lauchli, 1990; Cramer *et al.*, 1990; Ashraf and O'Leary, 1994). Higher K requirements for plants exposed to drought and salinity stress (Chow *et al.*, 1990) appear to be due to the need to maintain high potassium concentration under these conditions. High salt (NaCl) uptake competes with the uptake of

other nutrient ions, especially K^+, leading to K^+ deficiency. Epstein (1966) showed that there is an antagonistic relationship between K and Na uptake. This antagonism may be due to the direct competition between K and Na at a site of ion uptake in the plasmalemma. Na may also enhance the efflux of K into the growth medium, because of disturbance in membrane integrity (Cramer *et al.*, 1985). Under saline condition plant cell utilize K^+ as a metabolite to maintain turgor to escape from osmotic shock (Greenway and Munns, 1980; Blum, 1988). Islam (2001a) and Raptan *et al.* (2001) reported in mungbean and blackgram that potassium deficiency occurred due to salinity. Increased treatment of NaCl induces decrease in K^+ levels in a number of plants (Khan *et al.*, 1999, 2000a; Khan, 2001). Salinity decreases the ratio of K^+/Na^+ in *Vicia faba* (Gadallah, 1999).

Studies have indicated that the primary effect of salt stress is a disruption of membrane integrity caused by the displacement of Ca^{2+} from the cell surface by Na^+ (Cramer *et al.*, 1985; Lynch *et al.*, 1987; Suresh *et al.*, 1991). Cramer *et al.* (1987) demonstrated evidence for displacement of membrane-associated Ca^{2+} by Na^+ in root hairs of salinized cotton (*Gossypium hirsutum* L.) seedlings. They found that high concentrations of Na^+ displaced Ca^{2+} from plasma membrane. The displacement of Ca^{2+} inhibited the transport of ions into the root (Cramer *et al.*, 1987, 1989) and up to the top of the shoot (Lynch and Lauchli, 1985). High calcium levels were found to protect the cells of the maritime halophyte, *Aster tripolium* L. from adverse effects of salinity (Perera *et al.*, 1995). Salinity induced reduction in the calcium uptake was observed by Nakamura *et al.* (1990) in mungbean, Patil *et al.* (1992) and Patil *et al.* (1995) in greengram. Calcium concentration in the shoots decreased with increasing salinity (Al-Zaharani and Hajar, 1998). Increased treatment of NaCl induces decrease in Ca^{2+} levels in a number of plants (Khan *et al.*, 1999, 2000a; Khan, 2001).

Application of 0.5mM SA treatment had significant effect in restricting the low accumulation of nitrogen, phosphorus, potassium and calcium concentrations compared with control (Tables 39-42,62-65). The favourable effects of 0.5mM SA under 50mM NaCl were seen. In Pusa Vishal (mungbean), at 20DAS, application of 0.5mM SA increased potassium and calcium concentrations of plants grown under 50mM NaCl which was higher than control. However, in Tram (mungbean) the increase was noted only for potassium concentration at early growth stage. At 40DAS, nitrogen, potassium and calcium concentrations were found higher than control with the application of 0.5mM SA on Pusa Vishal (mungbean) plants treated with 50mM NaCl. In Tram (mungbean), only potassium concentration was found increased with 0.5mM SA of 50mM NaCl treated plants (Tables 39,41-42). In both the cultivars of mustard, at 30DAS, nitrogen and potassium concentrations were increased in comparison to the respective controls. The application of 0.5mM SA under saline condition also increased the calcium concentration in Alankar (mustard). At 60DAS, the treatment of 0.5mM SA on Alankar (mustard) enhanced the nitrogen, potassium and calcium concentrations of plants grown under 50mM NaCl. In PBM16 (mustard), only two characteristics nitrogen and potassium concentrations increased at 60DAS. At 90DAS in Alankar, the nitrogen, potassium and calcium concentrations increased over control with the application of 0.5mM SA on plants treated with 50mM NaCl. In PBM16, the increase was found only in nitrogen and potassium concentrations (Tables 62,64-65). Angrish *et al.* (2001) reported that amelioration of salinity was due to enhanced nitrogen status through presowing wheat seeds with plant growth regulators. SA treatments significantly increased total nitrogen concentration of *Zea mays* plants grown in saline conditions (Gunes *et al.*, 2005, 2007). SA pretreatments (10^{-7}–10^{-4}M) reduced K^+ contents in leaves of tomato under salt stress (Szepesi *et al.*, 2005). Al-Hakimi and Hamada (2001) suggested that grain soaking in SA could counteract the adverse effects of NaCl salinity on

potassium and calcium uptake of wheat seedlings. Maize plants receiving SA increased phosphorus and potassium concentrations in stress conditions (Gunes *et al.*, 2005).

Pretreatment of mungbean and mustard leaves with SA alleviated the inhibitory effects of salinity stress. In conclusion, high nutrient uptake (N, P, K and Ca) and low toxic ion uptake (Na and Cl) due to SA application reduced the salinity effects and increased dry matter accumulation in both the tolerant and non-tolerant types of mungbean and mustard. The results of this study signify the role of SA in alleviating the stress response of mungbean and mustard, and suggest that SA could be used as a growth regulator to improve plant growth and nutrient utilization under saline stress conditions. The results of this study give new insight on the SA-mediated ion accumulation and the control of salinity stress. SA treatment of salt-stressed mungbean and mustard plants could stimulate their salt tolerance by accelerating nitrogen accumulation. The study on the influence of SA on the enzymes of nitrogen metabolism, however, is required to strengthen the information on SA-stimulated nitrogen accumulation and subsequent effect on salinity tolerance. Similarly, the studies on the SA stimulated phosphorus, potassium and calcium are needed for their role in salinity tolerance.

5.3.4 Activities of antioxidative enzymes

Salt stress causes oxidative stress because of water deficit and increasing ionic and osmotic effects. As a result of oxidative stress, the formation of active oxygen species, superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals (OH^-) and H_2O_2 takes place. The active oxygen species has been found to be the by-products of hyperosmotic and ionic stresses that causes membrane disfunction and damage to wide variety of metabolic activities (Bohnert and Jensen, 1996).

Plants possess a wide array of defense strategies to protect itself from active oxygen species (Foyer and Harbinson, 1994). Production of antioxidative enzymes is one part of the defense system that plants require to

protect against stress. Superoxide dismutase (SOD) constitute the primary step of cellular defense. It dismutates $O_2^{\bullet -}$ to H_2O_2 and O_2. Further, the accumulation of H_2O_2 is restricted through the action of catalase (CAT) or by the ascorbate-glutathione cycle, where ascorbate peroxidase (APX) reduces it to H_2O. Finally, glutathione reductase (GR) catalyzes the NADPH-dependent reduction of oxidized glutathione to the reduced glutathione (Noctor *et al.*, 2002).

In the present investigation, the activities of antioxidative enzymes, CAT, SOD, APX and GR were enhanced in the presence of 50mM NaCl. The application of 0.5mM SA further enhanced the activities. The activities of the antioxidative enzymes were greater in tolerant cultivars than non-tolerant cultivars of mungbean and mustard (Tables 43-44,66-67). It may be argued that the tolerant cultivars have constitutively higher activities of antioxidative enzymes and inductively by 0.5mM SA application. Therefore, SA-treated tolerant cultivars of mungbean and mustard not only reduced the adverse effects of salinity, but even enhanced the values for a characteristics over control by efficiently detoxifying active oxygen species and protecting membrane permeability and photosynthetic apparatus and increasing dry matter production.

Reports on the increase in the activities of antioxidative enzymes due to salt stress are available in the literature, which depends on the plant type, growth conditions and the organ assayed. Hernandez *et al.* (2000) have reported that APX, GR, MDHAR, DHAR and SOD increased in wheat under salt stress. In wheat, Meneguzzo and Navarilzzo (1999) found differential antioxidative enzyme activities in shoot and root. The activities of enzymes increased in shoot and decreased in roots.

A study on *B. parviflora* showed decrease in CAT activity, while other enzymes like SOD and GR increased under salinity stress (Parida *et al.*, 2004c). In rice, cucumber and wheat also, the decrease in CAT activity has

been observed due to salinity stress (Shim *et al.*, 2003). The decrease in the activity was more severe with time after the salt treatment, even though the constitutive activity in control and the rate of decrease by salt stress were different among the plant species. The CAT activity was found decreased more severely in salt sensitive rice and cucumber compared with wheat (Shim *et al.*, 2003).

In contrast, studies have shown that CAT activity increased with salt stress in rice (Lin and Kao, 2000) and cucumber (Lechno *et al.*, 1997). This indicated that the responses might be different that depended on the intensity of the stress, plant part, time assayed after stress treatment. In my studies of mungbean and mustard, salt stress increased the catalase activity. In this study, application of 0.5mM SA increased the CAT activity under both saline and non-saline conditions (Tables 43,66). On the similar lines, Popova *et al.* (2003) reported that treatment of barley plants with 500μM SA or pretreatment with SA before exposure to 10μM paraquat increased CAT activity. SA treatment has also been found to increase CAT activity during chilling stress (Kang *et al.*, 2003a, b), and after 1d recovery the enzyme activity reached the control level (Pal *et al.*, 2002).

CAT appears to play an important role in SA induced stress tolerance, as it binds SA *in vitro* (Chen *et al.*, 1993b). The activity of CAT has been found inhibited by SA in several plant species (Sanchez-Casas and Klessig, 1994; Conrath *et al.*, 1995). However, there are doubts as to whether this binding is of biological importance since it seems that it is not specific to catalase, but is a general feature of all iron-containing enzymes (Ruffer *et al.*, 1995).

In the reported study, treatment of 50mM NaCl increased the SOD activity in both tolerant and non-tolerant cultivars of mungbean and mustard (Tables 43,66). The increase in SOD activity due to salt stress has also been reported by Kalir and Poljakoff-Mayber (1981), Kandpal and Rao (1982), Kalir *et al.* (1984) and Mishra *et al.* (1995). It may be said that high levels of SOD

activity are essential for survival of plants under salinity stress conditions for dismutation of superoxides. Thus, the activity of SOD in salt tolerant cultivars, Pusa Vishal and Alankar was higher than the sensitive cultivars, Tram and PBM16. It has been demonstrated that salt tolerant cotton (Gossett *et al.*, 1994), barley (Acar *et al.*, 2001), tomato (Shalata and Tal, 1998), and wild beet (Bor *et al.*, 2003) exhibited higher levels of SOD as compared to their salt sensitive cultivars. In contrast, Dionisio-Sese and Tobita (1998) found low level of SOD in salt tolerant cultivars than sensitive cultivars. This behaviour of low SOD activity of tolerant cultivars exposed to NaCl, has been partly attributed to the lower Na^+ content in the leaves (Dionisio-Sese and Tobita, 1998).

The application of 0.5mM SA under non-saline conditions increased the activity of SOD in both the cultivars significantly. Application of 0.5mM SA on plants treated with 50mM NaCl exhibited higher increase in the SOD activity compared with the increase observed in 50mM NaCl treatment (Tables 43,66). SA treatment has been shown to increase SOD activity of plants under various kinds of stress. Pretreatment with SA before paraquat application enhanced the activity of SOD by 20%, compared with both control and paraquat-treated leaves (Popova *et al.*, 2003). The increase in SOD activity was observed in both SA-free and SA-primed roots (Choudhury and Panda, 2004). Kang *et al.* (2003a, b) found an increase in SOD activity with SA under chilling stress, but was insensitive to SA treatment under normal growth conditions. They further showed that accumulation of H_2O_2 in SA pretreated banana leaves before chilling stress might refer to the lower scavenging ability to H_2O_2 by the stable SOD activity. Popova *et al.* (2003) reported that no changes were observed in the activity of SOD in barley seedlings treated with SA and kept for 24h in the dark. However, 6h after light exposure and treatment with 500µM SA caused an increase in SOD activity by 17%.

The other antioxidative enzyme, GR in both the cultivar types increased significantly with 50mM NaCl (Tables 44,67). The activity of GR was

significantly further increased when 50mM NaCl was supplemented with 0.5mM SA. Similar finding has also been reported by Szalai *et al.* (2005). They found GR activity increased in leaves treated with 100mM NaCl after 1d but in the roots only after the 4d recovery in maize plants (Szalai *et al.*, 2005). GR enzyme is found to be induced by stress treatments (Mishra *et al.*, 1995). In a study on *Arabidopsis*, NaCl treatment decreased the GSH/GSSG ratio by approximately 91% in wild type, whereas in NahG *Arabidopsis* seedlings the GSH/GSSG ratio decreased by 71% approximately after exposure to NaCl (Borsani *et al.*, 2001). The transgenic plants overexpressing GR had shown both elevated levels of GSH and increased tolerance to oxidative stress in leaves (Broadbent *et al.*, 1995). Addition of chemical agents that reduce active oxygen species levels also reduces the damaging effect of salt and osmotic stress, supporting the hypothesis that increased active oxygen species is the primary cause of the seedling lethality under stress conditions.

SA treatment increased the activity of GR of both cultivars significantly (Tables 44,67). In tolerant and non-tolerant cultivars of mungbean and mustard, the increase in the GR activity due to the combined treatment 0.5mM SA plus 50mM NaCl was more compared with the treatment of 50mM NaCl alone. An increase of about 20% in GR activity occurred in leaves treated with SA compared to the control leaves after 24h in the dark (Popova *et al.* 2003). GR activity was increased to two-fold by 1d of SA or SA+Cd treatment followed by 1d of recovery. However, when Cd was added after pretreatment with SA, the GR activity remained at the same high level. GR activity was increased by 0.5mM SA treatments to two-fold, and this high activity was maintained after 1d of recovery following the SA treatment as well (Pal *et al.*, 2002). In my study, 0.5mM SA application also increased GR activity in tolerant and non-tolerant cultivars. SA application also proved effective in increasing GR activity under other kind of stress. Pretreatment of SA before exposure to paraquat and light had a protective effect on the GR activity (Popova *et al.*,

2003). SA activated GR activity, which inturn increased chilling tolerance in subsequent 2°C stress (Janda *et al.*, 1999, 2000).

The activity of APX was found increased with 50mM NaCl in both the tolerant and non-tolerant cultivars (Tables 44,67). Application of 0.5mM SA increased the activity of APX of plants grown under non-saline or salinized conditions. Szalai *et al.* (2005) reported that there were no changes in the APX activity treated with 100mM NaCl at initial stage of growth i.e. 7d of maize plants. Application of 0.5mM SA increased the activity of APX of plants grown under 50mM NaCl which was higher than control. Pal *et al.* (2002) found that APX activity increased by about two-fold by SA and this increase did not change when SA pretreatment was followed by 1d of recovery or Cd treatment. However, when SA and Cd were added at the same time, their effect was synergistic, resulting in a three-fold increase in APX activity. However, contrary to this Popova *et al.* (2003) observed no significant changes in APX activity in leaves treated with 500μM SA for 24h in the dark. SA increased the activity of APX and improved the photosynthesis during chilling stress (Kang *et al.*, 2003a, b). The inhibition of APX by SA (Durner and Klessig, 1995) provided the first indications of the existence of a link between SA and the oxidative burst. SA also inhibited APX activity, increasing H_2O_2 level in treated tobacco leaves (Wendehenne *et al.*, 1998). Enhanced activity of enzymatic antioxidants by SA treatment has also been demonstrated by other workers (Dat *et al.*, 1998a; Gossett *et al.*, 2000; Kang and Saltveit, 2001).

5.3.5 Yield characteristics

Salt stress (50mM NaCl) led to a significant reduction in yield characteristics (discussed in the section 'Comparison of Cultivars Performance under Salinity Stress'). Yield characteristics were increased by SA application and was greater in non-saline (0mM NaCl) than in salinized conditions (50mM NaCl). Under 0mM NaCl, the yield characteristics in both the cultivars increased significantly with 0.5mM SA application. Application of 0.5mM SA

alleviated the inhibition of yield characteristics under saline conditions (50mM NaCl) (Tables 45-46,68-69; Figures 10,13). Arfan *et al.* (2007) reported in spring wheat cultivars, that exogenous application of 0.75mM SA improved the grain yield, number of grains and number of spikelets per spike of tolerant cultivar under non-saline conditions. However, 100-grain weight of tolerant cultivar was improved by 0.25 and 0.50mM SA application through the rooting medium under non-saline conditions. In contrast, in non-tolerant cultivar only number of grains was increased due to 0.75mM SA application under non-saline conditions. However, salt-induced reduction in grain yield, 100-grain weight and number of grains was considerably ameliorated in tolerant cultivar due to the application of 0.25mM SA. In contrast, grain yield of non-tolerant cultivar was slightly improved with 0.50mM SA application under saline conditions. Beneficial effect of SA application depends on type of species or cultivar.

The precise role of SA in improving yield is not evident. It can be stated that the beneficial effect of SA on yield may have been due to translocation of more photoassimilates to seeds during filling, thereby increasing seed weight. The second possible mechanism of SA-induced yield enhancement might be an increase in the number of pods and number of seeds, because SA has the capacity to both directly or indirectly regulate yield, e.g., boll number in cotton (Hampton and Oosterhuis, 1990) were found to be up-regulated by SA application. Gunes *et al.* (2007) reported that exogenous levels of SA increased dry yield of maize significantly both under saline and non-saline conditions. However, this effect of SA was more pronounced in saline conditions. In saline conditions with the highest level of applied SA (1.0mM), dry yield increased almost up to the yield obtained from the non-saline control treatment. In cheena millet (*Panicum miliaceum* L.), SA increased grain number (Datta and Nanda, 1985). In mungbean (*Vigna radiata* L.), foliar sprays of 7.2 and 72μM SA increased seed yield per plant by 19 and 46%, respectively (Singh and Kaur,

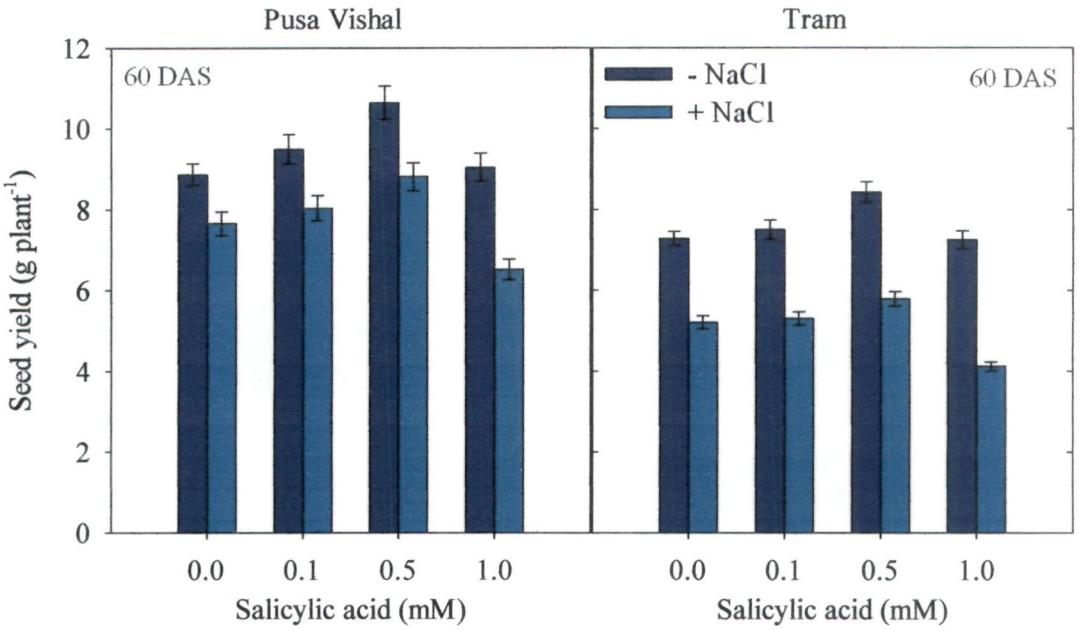


Figure 10: Effect of increasing concentrations of SA and salinity stress on seed yield of Pusa Vishal (salinity tolerant) and Tram (salinity non-tolerant) cultivars of mungbean (*Vigna radiata* L.) at harvest, i.e., 60 days after sowing (DAS). Data shown are mean ± S.E.

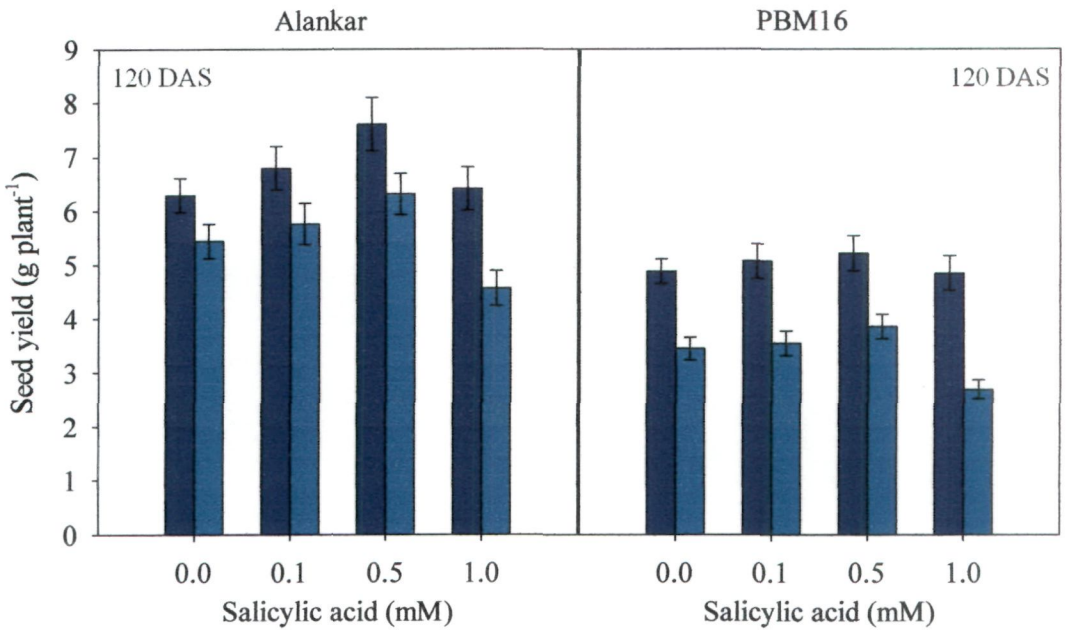


Figure 13: Effect of increasing concentrations of SA and salinity stress on seed yield of Alankar (salinity tolerant) and PBM16 (salinity non-tolerant) cultivars of mustard (*Brassica juncea* L.) at harvest, i.e.,120 days after sowing (DAS). Data shown are mean ± S.E.

1980). Zhou *et al.* (1999) reported that maize plants stem injected with SA produced 9% more grain weight than those with the control. An increase in number of pods has been found in *P. vulgaris* (Rendon, 1983; Lang, 1986) and wheat (Lopez, 1989) and an increase in yield has been found in barley (Pancheva *et al.*, 1996), maize (Zhou *et al.*, 1999; Khan *et al.*, 2003; Khodary, 2004) and soybean (Kumar *et al.*, 2000; Khan *et al.*, 2003).

It may be concluded that NaCl treatment decreased growth, photosynthetic and yield characteristics of tolerant and non-tolerant cultivars of mungbean and mustard. The salt treatment caused an accumulation of sodium and chloride to a higher extent and the essential nutrients, nitrogen, phosphorus, potassium to a lesser extent. The tolerant cultivars exhibited lesser decrease than the non-tolerant cultivars of both the crops when treated with NaCl. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean and mustard. In tolerant and non-tolerant cultivars, the increases were greater under non-saline (control) conditions than under saline conditions. The positive effect of 0.5mM SA application was found as it decreased sodium and chloride concentrations in both tolerant and non-tolerant cultivars under saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars increased significantly with SA application under both saline and non-saline conditions. Application of 0.5mM SA helped to reduce the adverse effects of salinity in both the crops. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl.

In tolerant and non-tolerant cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control for few characteristics. Salt-induced reduction in growth and finally yield characteristics in mungbean and mustard was improved by the foliar application of 0.5mM SA in both tolerant and non-tolerant cultivars. This

improvement in the above characteristics due to SA was associated with improved photosynthetic capacity. The changes in net photosynthetic rate due to SA application were due to metabolic factors, other than photosynthetic pigments and leaf carotenoids. The tolerant cultivars exhibited higher growth and photosynthetic traits than non-tolerant cultivars under saline conditions, which could explain the ability of salt tolerant cultivars to show better yield characteristics under salt stress than non-tolerant cultivars. SA also maintained higher activities of antioxidative enzymes under salt stress and better synergy among the enzymes helped to reduce the active oxygen species level and damage caused by it. It may be suggested that 0.5mM SA could be used as a potential growth regulator to improve plant growth, photosynthetic and yield characteristics under salt stress.

5.4 Future Research

Salinity is a limiting environmental factor for plant production, and is becoming more prevalent in agricultural soil due to several reasons. The study reported in the thesis shows that maximum reduction in the growth and photosynthetic characteristics were noted with 100mM NaCl in all the cultivars of mungbean and mustard. The treatment of 100mM NaCl was so intense that it proved detrimental on yield characteristics and the plants could not survive. SA plays an important role in abiotic stress tolerance, and considerable interests have focused on SA due to its ability to induce a protective effect on plants under stress. Plants respond to stress by the synthesis of signaling molecules. These activate a range of signal transduction pathways. Several such signaling molecules have been identified in plants. The study of interaction of these molecules with SA may provide fruitful information on the influence of SA on plants under normal conditions and its potential in alleviating salt stress effects. The signaling molecules may be ABA, calcium, jasmonic acid and ethylene. High salt concentration triggers an increase in levels of plant hormones such as ABA. ABA is responsible for the alteration of salt-stress genes. ABA has been

found to alleviate the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates. ABA promotes stomatal closure by rapidly altering ion fluxes in guard cells under stress conditions. Other ABA actions involve modifications of gene expression, and the analysis of ABA-responsive promoters has revealed diversity of potential cis-acting regulatory elements. The nature of the ABA receptors remains unknown. The combined biophysical, genetic and molecular approaches have led to considerable progress in the characterization of more downstream signaling elements. In particular, substantial evidence points to the importance of reversible protein phosphorylation and modification of cytosolic calcium levels and pH as intermediates in ABA signal transduction. Increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term. Jasmonates also have important roles in salt tolerance. Jasmonates are generally considered to mediate signaling, such as defense responses, flowering, and senescence. However, factors involved in the jasmonate signal-transduction pathway remain unclear. Ethylene is now considered as a plant hormone regulating growth and photosynthetic responses in plants. Evidences indicate that it plays a prominent role in managing abiotic stress (Druege, 2006). Further study on the interaction of SA, ABA and ethylene may provide in-depth insight into the role of SA in alleviating salt stress.

SUMMARY

SUMMARY

The present thesis entitled "Physiological Basis for the Salicylic Acid-Mediated Tolerance of Mungbean (*Vigna radiata*) and Mustard (*Brassica juncea*)" comprises of six chapters.

In Chapter 1, the importance of the problem and justifications for the present work undertaken were emphasized.

Chapter 2 is the review of literature. It deals with the relevant literature on the aspects of salinity stress and salicylic acid. The chapter has been divided in sections and sub-sections for better understanding of the work of other researchers in this field of study.

Chapter 3 describes the details of the materials used in the study and methodology adopted to determine various characteristics recorded in the four experiments. Relevant information on the experimental design, location of the study and the environmental conditions during the data sampling times has been mentioned.

Chapter 4 includes the results on crop responses to treatments in the four experiments. The results were statistical analysed and the significance at $P<0.05$ was determined. The treatment means were separated by the Duncan's Multiple Range Test (DMRT).

In Chapter 5, results have been discussed in the light of observations recorded and supported with the earlier findings, if available on the subject. Possible explanations of the data obtained have also been included to reach a conclusion. The results of the experiments are summarized below:

Experiments 1 and 2

Experiment 1 was conducted on mungbean and Experiment 2 on mustard to assess the effects of salinity stress (0, 50 and 100mM NaCl) on four cultivars. The purpose of the study was to select tolerant and non-tolerant cultivars on the basis of their growth, photosynthetic and yield characteristics at

20 and 40DAS in mungbean and at 30, 60 and 90DAS in mustard. Yield characteristics were determined at 60DAS in mungbean and 120DAS in mustard. The design of the experiments was randomized block design. Growth characteristics determined were : root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass. Photosynthetic characteristics were : carbonic anhydrase activity and net photosynthetic rate. At harvest, yield characteristics determined were : pod length, pod number per plant, seed number per pod and seed yield.

Experiment 1 (2003): Maximum reductions in the growth and photosynthetic characteristics were noted with 100mM NaCl at 20 and 40DAS in all the cultivars of mungbean. Upto maturity stage, treatment of 100mM NaCl proved deleterious and plants did not survive in this treatment. Plants treated with 50mM NaCl, thus exhibited a significant and maximum decrease over control on yield characteristics. Therefore, 50mM NaCl concentration was considered suitable to assess tolerance of the cultivars, and this concentration was used in further experiments. The cultivar Tram exhibited maximum decrease followed by T44 whereas Pusa Vishal registered lowest decrease followed by PBM54. The order of the tolerance of the cultivars to salinity stress was Pusa Vishal > PBM54 > T44 > Tram.

Experiment 2 (2003-2004): The effect of 100mM NaCl decreased the growth and photosynthetic characteristics maximally and was more conspicuous on all the cultivars of mustard at 30, 60 and 90DAS sampling times. However, the effect of 100mM NaCl on yield characteristics was detrimental and the plants could not survive. The cultivars, Alankar and Pusa Bold had significantly more growth, photosynthetic and yield characteristics than Sakha and PBM16 under 50mM NaCl concentration. The order of the suitability of the cultivars to salinity stress in terms of growth, photosynthetic and yield characteristics was Alankar > Pusa Bold > Sakha > PBM16.

Experiments 3 and 4

Experiments 3 and 4 were conducted based on the findings of Experiments 1 and 2. The aim of the experiments was to study the effects of exogenous application of salicylic acid in alleviating salinity stress and the physiological processes associated changes with the salicylic acid treatment on tolerant and non-tolerant cultivars of mungbean (Experiment 3) and mustard (Experiment 4). From the results of Experiment 1, Pusa Vishal and Tram cultivars of mungbean were categorized as tolerant and non-tolerant cultivars, respectively. Similarly, from Experiment 2 it was clear that Alankar and PBM16 cultivars of mustard were tolerant and non-tolerant, respectively. This has also been detailed out in earlier pages that the treatment of 100mM NaCl was deleterious for both the crops. Therefore, the plants were burned at maturity stages of the crops. Both the experiments (3 and 4) were confined with the use of 0 or 50mM NaCl for growing plants and the application of 0.0, 0.1, 0.5 and 1.0mM SA on foliage at 15DAS on tolerant and non-tolerant cultivars was studied on ameliorating salt stress effects. In these two experiments growth, photosynthetic, biochemical and yield characteristics were studied. The time of sampling for these characteristics for mungbean was 20, 40 and 60DAS, and 30, 60, 90 and 120DAS for mustard. The activities of antioxidative enzymes were also studied in both the crops at first sampling time. Growth characteristics were similar as in earlier experiments. Photosynthetic characteristics were : carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and the contents of chlorophyll and carotenoid. Biochemical characteristics were : concentration of sodium, chloride, nitrogen, phosphorus, potassium and calcium. The activities of antioxidative enzymes assayed were : catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase. Yield characteristics were similar as in Experiments 1 and 2. The design of the experiments was randomized block design.

Experiment 3 (2004): Salt stress led to a significant reduction in growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of both the cultivars. The cultivar Tram exhibited a higher reduction than Pusa Vishal. The treatment of 50mM NaCl increased sodium and chloride concentrations in Pusa Vishal and Tram at 20 and 40DAS, and the accumulation was higher in Tram than Pusa Vishal. Exposure of plants to 50mM NaCl increased the activities of antioxidative enzymes in both the cultivars but to a higher degree in Pusa Vishal than Tram. The treatment of 0.5mM SA was found most effective in alleviating salinity stress on growth, photosynthetic, biochemical and yield characteristics. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean. In both the cultivars i.e. Pusa Vishal (tolerant) and Tram (non-tolerant) the increases were greater under non-saline (control) conditions than under saline conditions (50mM NaCl) at 20 and 40DAS. The positive effect of 0.5mM SA application was also found as it decreased sodium and chloride concentrations under both saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars further increased significantly with 0.5mM SA under both saline and non-saline conditions. In Pusa Vishal, at initial growth stage i.e. 20DAS, the application of 0.5mM SA increased potassium and calcium concentrations of plants grown under 50mM NaCl which was higher than control. However, in Tram, the increase was noted only for potassium concentration. At later growth stage, photosynthetic characteristics, nitrogen, potassium and calcium concentrations were found higher than control with the application of 0.5mM SA on Pusa Vishal plants treated with 50mM NaCl. In Tram, only potassium concentration was found increased with 0.5mM SA of 50mM NaCl treated plants.

Experiment 4 (2004-2005): Application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations, activities of antioxidative enzymes and yield characteristics of Alankar (tolerant) and PBM16 (non-tolerant) cultivars grown under non-saline (control) conditions. Non-salinized plants treated with 0.5mM SA maintained a higher growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics than salinized plants at all the stages, indicating adverse effect of the NaCl salinity in tolerant (Alankar) as well as non-tolerant (PBM16) cultivars. Application of 0.5mM SA decreased the concentrations of sodium and chloride in both tolerant (Alankar) and non-tolerant (PBM16) cultivars, under normal and saline conditions at all the sampling times. Growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics decreased significantly with 50mM NaCl in both the cultivars but more adverse effects of salinity were found on PBM16. However, the concentrations of sodium and chloride and the activities of antioxidative enzymes increased with 50mM NaCl in both the cultivars. Application of 0.5mM SA helped to reduce the adverse effects of salinity. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl. In both the cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control at 30DAS. Nitrogen and potassium concentrations and activities of antioxidative enzymes were increased in comparison to the respective control. The application of 0.5mM SA under saline conditions also increased the calcium concentration in Alankar. At 60DAS, the treatment of 0.5mM SA on Alankar enhanced the photosynthetic characteristics, nitrogen, potassium and calcium concentrations of plants grown under 50mM NaCl. In PBM16, only two characteristics nitrogen and potassium concentrations increased at 60DAS. At 90DAS, in Alankar, the growth and photosynthetic characteristics, nitrogen, potassium and

calcium concentrations increased over control with the application of 0.5mM SA on plants treated with 50mM NaCl. In PBM16, the increase was found only for nitrogen and potassium concentrations. In Alankar, the yield characteristics were found higher than control with 0.5mM SA application on plants treated with 50mM NaCl.

The present chapter is followed by an up-to-date bibliography of the literature cited in the text.

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